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CONTRACTING ORGANIZATION: University of California San Francisco

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14. ABSTRACT

One of the reasons for the lack of successful clinical trials for spinal cord injury (SCI) is the lack of biomarkers for patient stratification. We have recently proposed a novel approach for the discovery of white blood cell (WBC) RNA biomarkers. Our approach yielded transcriptomic signatures in human WBCs that accurately diagnose SCI severity. Our hypothesis is that these gene modules are also functionally involved in the pathophysiology of SCI and could be targeted for the development of therapeutics. We performed Gene Co-Expression Network Analysis on RNAseq data from WBCs in humans and rats after SCI and identified gene modules associated with SCI severity in both species. Using homologenes, we identified conserved WBC gene modules with several of them being highly associated with SCI severity in both species. WBC transcriptomic biomarkers may potentially be used as targets for therapeutic interventions after SCI. Using evolutionarily conserved SCI-induced gene signatures significantly increases the probability for an effective preclinical model to be translatable in human SCI.

15. SUBJECT TERMS
Spinal cord injury, white blood cells, conserved transcriptomic signatures, biomarkers, injury severity.

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INTRODUCTION

Spinal cord injury (SCI) is a devastating condition that affects millions worldwide and adds thousands of newly injured patients to that pool annually. In the US only, 18,000 new SCIs happen annually, altering dramatically the lives of these new patients and their caretakers. Despite the clinical and research efforts over the past four decades, a therapy for SCIs is still lacking. This funded research aims to discover novel therapeutics using a systems biology approach where a preclinical model will be simultaneously validated by clinical research findings in a bedside-to-bench and back approach. We will use acute transcriptomic signatures in the white blood cells (WBCs) of humans and rats significantly associated with the initial SCI severity and/or long-term functional recovery. From this global transcriptomic pattern of both species, we will derive homologenes to be used to discover evolutionarily conserved transcriptomic signatures associated with SCI severity and recovery in both species. These signatures will subsequently be used in the public database CMap to bioinformatically predict compounds from a list of more than 8,000 perturbagens that could reverse the conserved transcriptomic phenotype. The core hypothesis is that the reversal of the expression phenotype of these signatures will lead to improved long-term recovery. The hypothesis will be tested *in vivo* in a preclinical (rat) model of SCI after the top-ranked predicted compounds will be first validated *in vitro* in rat WBC primary leukocyte cultures. A successful *in vivo* drug trial will have a great chance of being clinically important and applicable in human SCI since the discovered drug will be, by definition (conserved signature), targeting a transcriptomic signature behaving similarly in humans after SCI.

KEYWORDS

Spinal cord injury, white blood cells, conserved transcriptomic signatures, biomarkers, drug discovery, injury severity.

ACCOMPLISHMENTS

What were the major goals of the project?

Per the approved Statement of Work (SoW) the goals that would be completed during the 2nd year of the funding period were:

Major Task 3: Identify species-specific gene modules in WBCs after SCI by analyzing consensus modules with respect to injury severity in humans (AIS grades, n = 90) and rats (impact force and behavioral score at 7 days post SCI; n = 42)	13-20	MO/NK
Subtask 3.1: analyze consensus modules with respect to injury severity in humans (AIS grades, n = 90) and rats (impact force and behavioral score at 7 days post SCI; n = 42)	13-15	MO/NK
Subtask 3.2: Correlate the WBC consensus modules with functional outcomes of long-term recovery in rats and human (the latter only if possible after addition of TRACK-SCI data throughout the PoP)	13-20	MO/NK
Subtask 3.3: Using the existing WBC RNA-seq databases converted in Subtask 2.1, perform differential co-expression analysis to identify whether there are rat-specific gene modules that are not found in humans, and vice versa (i.e., human-specific module identification).	16-18	MO/NK
Milestone(s) Achieved: RNA-seq datasets organized for cross-species comparison; Consensus modules with respect to species, injury severity, and lesion volume established and analyzed; differential co-expression analyses established; conference abstract or talk describing Aim 1 results submitted; paper describing conserved gene networks after SCI in humans and rats submitted		
Specific Aim 2: Discover conserved WBC-derived gene expression patterns that are preserved in the human and rat injured spinal cords		

Major Task 4: Identify gene expression patterns in the rat spinal cord of different severities at two different time points.	9-20	NK/MO
Subtask 4.1: Perform SCIs of three injury severities (naïve, sham, 75, 100, and 150 kdynes, n = 12 each), sacrifice half the animals at 48 hours post SCI and the other half at 6 weeks post SCI and extract total RNA from the injured hemicord.	9-15	NK
Subtask 4.2: Perform 3'Tag RNAseq on the spinal cord RNA samples and create the normalized gene count matrix for each sample.	16-18	NK
Subtask 4.3: Through gene co-expression network analysis identify spinal cord gene modules which correlate with the injury severity and the time point (acute vs. chronic).	19-20	MO/NK

Per the SoW the goals that would be initiated during the 2nd year of the funding period but would be completed during the 3rd year are:

Major Task 5: Identify gene expression patterns in the human spinal cord using postmortem spinal cord sections from donors and identify conserved and distinct modules between humans and rats.	13-26	NK/MO
Subtask 5.1: Extract total RNA from formalin fixed paraffin embedded postmortem human spinal cord sections from donors who had suffered from SCI (n = 11, request for tissue sections from ISCIB already approved).	13-15	NK
Subtask 5.2: Perform 3'Tag RNAseq on the human spinal cord RNA samples and create the gene count matrix for each sample.	16-18	NK
Subtask 5.3: Identify conserved gene expression modules in the spinal cord between humans and rats with the same analytical pipeline as in Major Task 2.	19-24	MO/NK
Subtask 5.4: Rank the WBC-derived conserved gene modules identified in Major Tasks 2 and 3 by their preservation score in the spinal cord of humans and rats.	25-26	MO/NK

What was accomplished under these goals?

During the 2nd year of the funded research we accomplished the following milestones:

1) Completed the rat experiment in which we harvested the injured spinal cords of three different severities at two different time points in rats. All animals were also assessed for functional outcomes.

2) RNA was extracted from the spinal cords and was sequenced using 3'Tag RNAseq.

3) RNA was extracted from human post-mortem FFPE spinal cord sections and was submitted to the sequencing facility

4) In vitro experiments were initiated to establish cell culture parameters for the drug screening.

We have recently completed our initial in vivo experiment, in which we conducted a C5 SCI on a cohort of 48 adult Long Evans Rats, consisting of both males and females. This experiment replicated the three injury severities (75, 100, and 150 kdynes) used in our previous work. Additionally, we included a control group of 12 adult Long Evans rats, on which we performed a laminectomy surgery at the C5 level, serving as trauma controls (sham).

Before the injuries were induced, all animals underwent a battery of three behavioral tests, including paw placement, grooming, and the Irvine-Beattie-Bresnahan forelimb digit test. Subsequently, assessments were conducted at various time points post-SCI, specifically at 2, 7, 14, 21, 28, 35, and 42 days post-injury. Half of

the animals were euthanized at the 2-day mark, immediately following their respective behavioral tests, while the remaining half were terminated at the 42-day mark. During termination, all animals underwent transcardial perfusion with ice-cold PBS, and we dissected a 1 cm section around the SCI epicenter. Following this, we split the spinal cord segment into two halves, mechanically dissociating the ipsilateral half in TRIZOL solution to extract total RNA. All RNA sample concentrations were found to be within the expected range. Subsequently, we submitted these samples to the UC Davis sequencing facility for 3'Tag-RNAseq and the analysis is nearly complete at this stage.

Concurrently, we received postmortem human FFPE tissue sections from 13 donors who had experienced spinal cord injuries through the International Spinal Cord Injury Biobank. These specimens arrived in excellent condition, and our objective was to extract total RNA from them. As this was a procedure unfamiliar to our lab, we utilized similar human FFPE tissue sections (placental tissue) generously provided by another UCSF lab for practice. We conducted tests using two different kits and explored various protocol variations. Ultimately, we succeeded in extracting sufficient RNA from the tissue sections. Presently, the RNA samples have been submitted to the UC Davis sequencing facility, and we anticipate receiving the results within the next 4-6 weeks.

Upon receipt of the human spinal cord RNAseq results, we will have all the necessary puzzle pieces to connect and identify evolutionarily conserved blood-derived transcriptomic signatures preserved in the spinal cord after injury. These signatures are expected to be correlated with injury severity and outcomes. With this data in hand, we will be well-prepared to leverage these signatures within the CMap platform to generate a list of candidate chemical compounds for targeting. In the interim, we have employed the CMap using only the signatures derived from conserved blood-derived profiles, and we have successfully generated the initial list of compounds. 103 compounds have passed all the statistical tests and below are the top 25 hits:

Score	ID	Name	Description	Target
-99.93	BRD-K05926469	lenalidomide	Antineoplastic	CRBN, TNF, CDH5, PTGS2, TNFSF11
-99.93	BRD-K65146499	nabumetone	Cyclooxygenase inhibitor	PTGS2, AKR1C3, CYP2B6, CYP2C19, CYP2E1, PTGS1
-99.89	BRD-M07438658	lapatinib	EGFR inhibitor	EGFR, ERBB2, CYP3A5
-99.89	BRD-K63195589	tipifarnib	Farnesyltransferase inhibitor	FNTA, FNTB
-99.89	BRD-K14329163	BAY-K8644	Calcium channel activator	CACNA1C
-99.79	BRD-K53561341	KIN001-220	Aurora kinase inhibitor	AURKA
-99.75	BRD-K63516691	T-0156	Phosphodiesterase inhibitor	PDE5A
-99.56	BRD-K19605405	ZM-241385	Adenosine receptor antagonist	ADORA2A, ADORA2B, ADORA1, ADORA3
-99.54	BRD-A45664787	iloprost	Platelet aggregation inhibitor	PTGIR, PTGER1, PTGER2, PDE4A, PDE4B, PDE4C, PDE4D, PLAT, PTGDR, PTGER3, PTGER4, PTGFR, TBXA2R
-99.51	BRD-K90382497	GW-843682X	PLK inhibitor	PLK1, PLK3
-99.48	BRD-A18202423	CPCCOEt	Glutamate receptor antagonist	GRM1
-99.44	BRD-A83855350	naltrexone	Opioid receptor antagonist	OPRK1, OPRM1, OPRD1, SIGMAR1

-99.43	BRD-K06817181	BRD-K06817181	JAK inhibitor	JAK2
-99.4	BRD-K78280988	anandamide	Cannabinoid receptor agonist	CNR1, CNR2, TRPV1, CACNA1G, CACNA1H, CACNA1I, GLRA1, GPR119, GPR18, GPR55, KCNA2, KCND3, KCNK3, KCNK9, TRPM8
-99.22	BRD-K71799949	carbamazepine	Carboxamide antiepileptic	ABCB1, CYP1A2, CYP3A4, SCN1A, SCN3A, SCN5A
-99.19	BRD-K61323504	SB-225002	CC chemokine receptor antagonist	CXCR2
-99.17	BRD-K87573634	propylpyrazole	Estrogen receptor agonist	ESR1
-99.16	BRD-A02759312	betaxolol	Adrenergic receptor antagonist	ADRB1, ADRB2
-99.12	BRD-K72264770	QW-BI-011	Histone lysine methyltransferase inhibitor	EHMT2
-99.01	BRD-K14550461	doxercalciferol	Vitamin D receptor agonist	VDR
-98.98	BRD-K09638361	SA-63133		CSNK1E, CSNK1A1, CSNK1D, CSNK1G2
-98.95	BRD-M47937986	cefatrizine	Bacterial cell wall synthesis inhibitor	
-98.77	BRD-K53123955	niridazole	Phosphofructokinase inhibitor	
-98.76	BRD-K85503079	perospirone	Dopamine receptor antagonist	DRD2, HTR2A, DRD3, DRD4, HTR2C
-98.76	BRD-A94543220	bifonazole	Sterol demethylase inhibitor	CYP2B6

It is intriguing that the majority of candidate compounds have never been employed in the context of SCI. Nevertheless, it is noteworthy that many of these compounds effectively target well-known immune pathways that have previously been linked to SCI.

Finally, as we were formulating the protocol for human FFPE RNA extraction, we concurrently initiated our initial in vitro experiments in preparation for the drug screening process. We used three rats without spinal cord injuries and directly obtained their blood (3-5 mL) via syringe from the heart. Subsequently, we isolated WBCs and placed them into 24-well plates, where they were incubated at 37°C with 5% CO_2 for a duration of 24 hours. It's noteworthy that over 80% of the cells exhibited viability (as determined by Trypan Blue staining) during the incubation period. With the impending finalization of the compound list, which will incorporate the human spinal cord RNAseq data, we are well-prepared to commence the in vitro drug screening process.

What opportunities for training and professional development has the project provided?

In addition to the points I discussed in last year's report, I have had the privilege of being invited to deliver oral presentations at a couple meetings and grand rounds over the past year, during which I presented this project. I am pleased to note that I have successfully persuaded numerous individuals within the field regarding the vital importance of conducting direct cross-species comparisons for the identification of therapeutic targets. This has subsequently led to invitations to review manuscripts for prestigious journals. More recently, I was honored with an invitation to evaluate a similar grant proposal submitted to the Swiss National Foundation, coming from a highly distinguished researcher in our field. Finally, with the help of this funded project in April I became a faculty member at UCSF at the assistant professor rank.

How were the results disseminated to communities of interest?

Nothing to report

What do you plan to do during the next reporting period to accomplish the goals?

As previously mentioned, our immediate priority is to complete the computational analysis as soon as we receive the RNAseq data for the human spinal cord. This analysis should be expedited, as we have all the necessary R scripts in place from previous analyses, requiring only minimal adjustments. Following this, our next steps involve identifying gene modules that are conserved and found in both blood-derived and spinal cord tissues, which are correlated with SCI severity and/or recovery. We will then utilize these modules as input data for the CMap platform to update our list of candidate drugs.

Subsequently, as outlined in our proposal, we plan to conduct in vitro screening of the top candidates. This screening aims to identify the most potent compound capable of reversing the expression of the identified gene modules. We will utilize this selected compound for our final in vivo experiment to determine whether it can lead to improved functional restoration after a spinal cord injury.

IMPACT

What was the impact on the development of the principal discipline(s) of the project?

The primary discovery during this reporting period centers around the preliminary compilation of potential drug candidates aimed at blood-derived conserved gene modules. We were both intrigued and excited to observe that the majority of these drugs have not been previously employed in the context of SCIs. Simultaneously, several of these drugs target well-established immune-related pathways that have been demonstrated to have a critical impact on the pathophysiology and progression of SCI. We maintain high hopes that once the final candidate list is established, it will serve as an invaluable resource for our field, with several of these compounds eventually undergoing testing by our team and other researchers.

What was the impact on other disciplines?

Nothing to report.

What was the impact on technology transfer?

Nothing to report.

What was the impact on society beyond science and technology?

Nothing to report.

CHANGES/PROBLEMS

Over the past year, we encountered several obstacles that slightly derailed our project's schedule. In July 2023, our department, along with our laboratory, relocated to the new UCSF building, Pride Hall. While Pride Hall offers an exceptional academic and research environment, the intricacies of the move compelled us to postpone several wet lab experiments. Furthermore, as the youngest faculty member in our department, I assumed several administrative responsibilities, which hindered our computational analysis efforts.

Additionally, our SRA, Kenneth Fond, who had been collaborating with me during the first year of the funding period, was accepted into medical school and consequently left the lab. The process of hiring a new SRA took nearly five months, contributing to the delays.

Nevertheless, our lab has now fully settled into this new collaborative scientific environment, and we are diligently making up for the lost time. I anticipate that we will soon regain our momentum and meet the final deliverables within the established timeline.

PRODUCTS

Publications, conference papers, and presentations

1. Oral presentation at the 61st ISCoS Annual Scientific Meeting in Vancouver, Canada, 15-18 September 2022. Evolutionarily conserved blood transcriptomic signatures as diagnostic biomarkers and pharmacological targets for spinal cord injury.
2. Oral presentation at the UCSF Department of Neurosurgery Grand Rounds, San Francisco, 4 September 2023. Leveraging blood transcriptomic signatures to develop biomarkers and identify pharmacological targets for SCI.
3. Oral and poster presentation at the International Symposium for Neural Regeneration in Stevenson, WA, 23-27 April 2023. Evolutionarily conserved blood transcriptomic signatures as diagnostic biomarkers and pharmacological targets for spinal cord injury.
4. Poster presentation at National Neurotrauma Symposium in Austin, TX, 25-28 June 2023. Evolutionarily conserved blood transcriptomic signatures as diagnostic biomarkers and pharmacological targets for spinal cord injury.
5. Invited speaker at the ISRT meeting in London, UK, 13-16 September 2023. Leveraging blood transcriptomic signatures to develop biomarkers and identify pharmacological targets for SCI

Website(s) or other Internet site(s)

Nothing to report.

Technologies or techniques

Nothing to report.

Inventions, patent applications, and/or licenses

Nothing to report.

Other Products

Nothing to report.

PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	*Nikolaos Kyritsis*
Project Role:	*Principal Investigator*
Researcher Identifier (e.g. ORCID ID):	*0000-0001-7801-5796*
Nearest person month worked:	4
Contribution to Project:	*Dr. Kyritsis has been involved in every experiment and oversees the entire project.*
Funding Support:	

Name:	*Kenneth Fond*
Project Role:	*Staff Research Associate*
Researcher Identifier (e.g. ORCID ID):	*0000-0002-9154-6599*
Nearest person month worked:	1
Contribution to Project:	*Mr. Fond was involved in animal caretaking, behavioral testing, RNA isolation, and data organization in this project.*
Funding Support:	

Name:	*Amity Lin*
Project Role:	*Staff Research Associate*
Researcher Identifier (e.g. ORCID ID):	*0000-0002-5433-3623*
Nearest person month worked:	1
Contribution to Project:	*Ms. Lin performed all animal spinal cord injuries and supervised the animal caretaking*

Funding Support:	

Name:	*Michael Oldham*
Project Role:	*Co-Investigator*
Researcher Identifier (e.g. ORCID ID):	*0000-0001-7633-6932*
Nearest person month worked:	1
Contribution to Project:	*Dr. Oldham shared code and supervised closely the identification of the conserved gene modules.*
Funding Support:	

Name:	*Adam Ferguson*
Project Role:	*Co-Investigator*
Researcher Identifier (e.g. ORCID ID):	*0000-0001-7102-1608*
Nearest person month worked:	1
Contribution to Project:	*Dr. Ferguson assisted in developing the experimental design of the rat study to ensure proper statistical analysis and he reviewed all statistical analysis.*
Funding Support:	

Name:	*Michael Beattie*
Project Role:	*Co-Investigator*
Researcher Identifier (e.g. ORCID ID):	*0000-0001-9463-3631*

Nearest person month worked:	1
Contribution to Project:	*Dr. Beattie provided his expertise and thoughts on the progress of the project through weekly 1-on-1 hourly meetings specifically dedicated to this project.*
Funding Support:	

Name:	*Jacqueline Bresnahan*
Project Role:	*Co-Investigator*
Researcher Identifier (e.g. ORCID ID):	*0000-0003-2243-7054*
Nearest person month worked:	1
Contribution to Project:	*Dr. Bresnahan was closely participating in the rat study design and was offering her expertise and knowledge on all aspects of animal surgery and caretaking.*
Funding Support:	

Name:	*Abel Torres-Espin*
Project Role:	*Co-Investigator*
Researcher Identifier (e.g. ORCID ID):	*0000-0002-9787-8738*
Nearest person month worked:	1
Contribution to Project:	*Dr. Torres-Espin reviewed and corrected when necessary all R scripts generated for this project. He also supervised the linear modeling approach to associate the conserved gene modules with SCI severity.*
Funding Support:	

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to report.

What other organizations were involved as partners?

Nothing to report.