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（海外特別研究員事業）
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海 外 特 別 研 究 員 最 終 報 告 書

独立行政法人日本学術振興会 理事長　殿

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海外特別研究員としての派遣期間を終了しましたので、下記のとおり報告いたします。
なお、下記及び別紙記載の内容については相違ありません。

記

1. 用務地（派遣先国名）用務地：メリーランド州ベセスダ（国名：アメリカ合衆国）

2. 研究課題名（和文）※研究課題名は申請時のものと違わないように記載すること。
慢性創傷滲出液の網羅的解析による創部痛バイオマーカーの同定とそのメカニズムの解明

3. 派遣期間：平成 30 年 4 月 1 日　～　令和 2 年 3 月 31 日

4. 受入機関名及び部局名
アメリカ国立衛生研究所　国立看護研究所

5. 所期の目的の遂行状況及び成果…書式任意　**書式任意（A4 判相当 3 ページ以上、英語で記入も可）**
（研究・調査実施状況及びその成果の発表・関係学会への参加状況等）
（注）「6. 研究発表」以降については様式 10－別紙 1～4 に記入の上、併せて提出すること。

Wound pain management for both acute and chronic wounds continues to pose major clinical issues, especially with regards to pain control. Management of wound pain is particularly challenging for cognitively impaired patients because validated objective pain assessment tools are not available for this clinical population. A biomarker that correlates with wound pain levels could be used as a therapeutic endpoint to optimize wound pain management. We have revealed that biochemical analysis of wound exudate could be used as an objective wound pain assessment tool [1]. Thus, a next logical step is to identify a promising wound pain biomarker candidate. We previously found that psychological stress-related hormone, adrenocorticotropic hormone (ACTH), induced mechanical hyperalgesia possibly through producing 5,6-epoxyeicosatrienoic acid, which is a wound pain biomarker candidate. This finding clarified a mechanism that perceived stress exacerbates wound pain. We reported these findings at the World Congress on Pain 2018 conducted at Boston, MA and in an academic journal [2,3], which generated some interest that allowed me to network with worldwide research experts in the field.

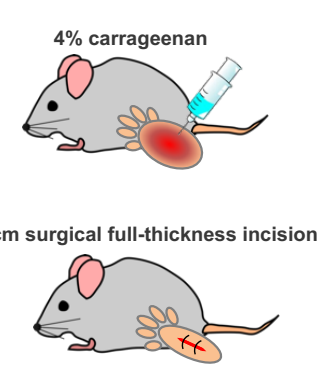

Figure 1 peripheral inflammation and surgical incision models. An injection of 150-microliter 4% carrageenan into left hind paw of rats was performed (A). A 1 cm incision on the left hind paw of rats was performed.

This ACTH related wound pain mechanism may be involved to wound pain chronification. Thus, next we have been interested in molecules that contribute to pain initiation because they can identify therapeutic targets for wound pain. We hypothesized that comparing molecular dynamics in

peripheral inflammation with wound will let us understand detailed mechanisms of inflammation, inflammatory and nociceptive pain, and tissue remodeling. We have been investigating the transcriptomic profiles of peripheral inflammation and surgical incision in extensively used rat models of pain and analgesia. The peripheral inflammatory rat model was established using an injection of 150-microliter 4% carrageenan into the left hind paw of the rats (Figure 1A). Similarly, a surgical incision rat model was established using a 1 cm incision made on the left hind paw of a different set of rats (Figure 1B). Tissue samples were obtained from the hind paw of both these models at 0 (naïve) to 12 days after the procedures (wounding or carrageenan injection) and subjected to histological analyses including hematoxylin and eosin staining, Masson's trichrome staining, and multi-plex in situ hybridization, and total RNA extraction for next-generation RNA sequencing (RNA-Seq). Thermal hyperalgesia and mechanical allodynia were detected using the Plantar Test Instrument (Ugo Basile, Comerio, Italy) and calibrated von Frey monofilaments, respectively. Statistical analyses used 2-way ANOVA with post hoc Sidak's test.

We measured hind paw thickness to assess hind paw edema in both inflammation and incision rat models. Statistical analyses showed that the thickness in the intervened sides were significantly greater than those in the control sides in the both models, indicating that both interventions induced hind paw edema. Furthermore, thermal hyperalgesia and mechanical allodynia were observed in the intervened sides in the both models. Using the datasets from the RNA-Seq data, we analyzed 3,817 differentially expressed genes (DEGs) in the inflammation model and 10,314 DEGs in the incision model. To cluster the DEGs, we used the DEGs between any two timepoints to construct a heatmap based on ratios of expression between timepoints, representing the time course of gene expression. The DEGs in the inflammation model were classified into 12 clusters (Figure 2A), and those in the incision model were classified into 14 clusters (Figure 2B). We hypothesized that each cluster may have each own distinct cell populations and biological functions. Thus, we are now deeply analyzing each cluster. The expression levels of genes encoding secreted pro-inflammatory cytokines, *Il6*, *Cxcl1*, *Cxcl2*, and *Il1b* (Figure 3A and C), as well as those related to immune response markers, *Cd68* (macrophage), *Ptprc* (B-cell), *S100a8* and *S100a9* (neutrophil) were strongly differential between the inflammation and incision models (Figure 3E and G). In addition, the peak of the expression patterns of these immune response genes were different. It was around 8 to 24 hours after the carrageenan injection in the inflammation model, and 24 to 72 hours after wounding in the incision model (Figure 3E and G). The multiplex in situ hybridization images validated the localization of each significant gene (Figure 3B, D, F, and H). These findings are consistent with the idea that important types of cells are similar, but different populations are involved in each model. These fundamental investigations build sets of objective readouts for wound and inflammation processes that may inform about pain status and which, in turn may guide clinical decisions regarding selection of the appropriate analgesic drug for wound pain management. These very important findings were reported as a poster presentation at: The Opioid Crisis and

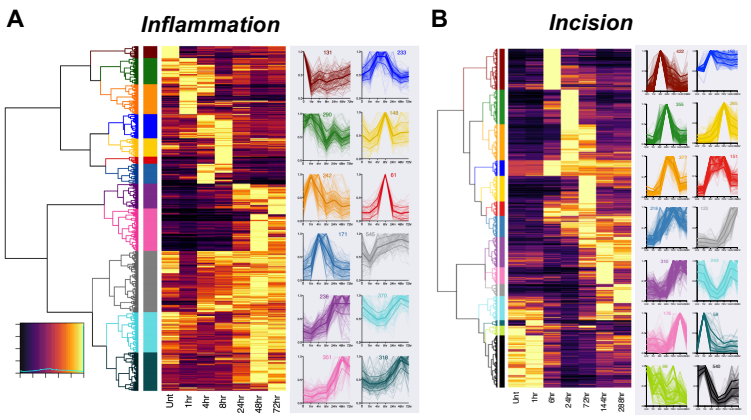

Figure 2 Clustering genes by expression patterns. These heatmap images indicate gene expression clusters of the peripheral inflammation and the incision models. The genes in the inflammation model were classified into 12 clusters, and those in the incision model were classified into 14 clusters.

the Future of Addiction and Pain Therapeutics: Opportunities, Tools, and Technologies Symposium conducted at the National Institutes of Health, Bethesda Campus, MD on Feb 2019 [4], the Wound Healing Society's 31st Annual Meeting to be held at San Antonio, TX in May 2019 [5], 14th Annual NIH Pain Consortium Symposium, Bethesda, MD in May 2019 [6], Symptom Science Center: A Resource for Precision Health, Bethesda, MD in Jun 2019 [7], National Advisory Council for Nursing Research [8], and the 4th Japan-US Science Forum in Boston 2019, Boston, MA in Nov 2019 [9]. Many researchers who attended the meetings were interested in my presentation and we had productive discussion for the next steps of this research. Further analysis of the RNA-Seq data is ongoing to assess additional inflammation-specific and incision-specific gene expression results. We have been examining genes encoding secreted proteins from the datasets to determine key regulators in the inflammatory model and the incision model. By analyzing orchestrated mechanisms associated with these differentially expressed genes, we can try to identify possible pathways and contributors to regulate hyperalgesia during the inflammatory response and to understand that some combination of these factors may be biomarkers for nociceptive and inflammatory pain. Moreover, we have been investigating anatomical tissue changes and remodeling along with transcriptional changes longitudinally during peripheral inflammation and surgical incision. We have found unique expression patterns of collagen genes in the inflammation model. These analyses may identify key biomarkers and therapeutic targets for wound healing. Now we have one manuscript that is under review by a high impact journal and are planning to publish two more research papers using these datasets.

Furthermore, we are running protocols to analyze wound samples obtained from human chronic wounds and acute wounds. As a continuous project of my post-doctoral research, which

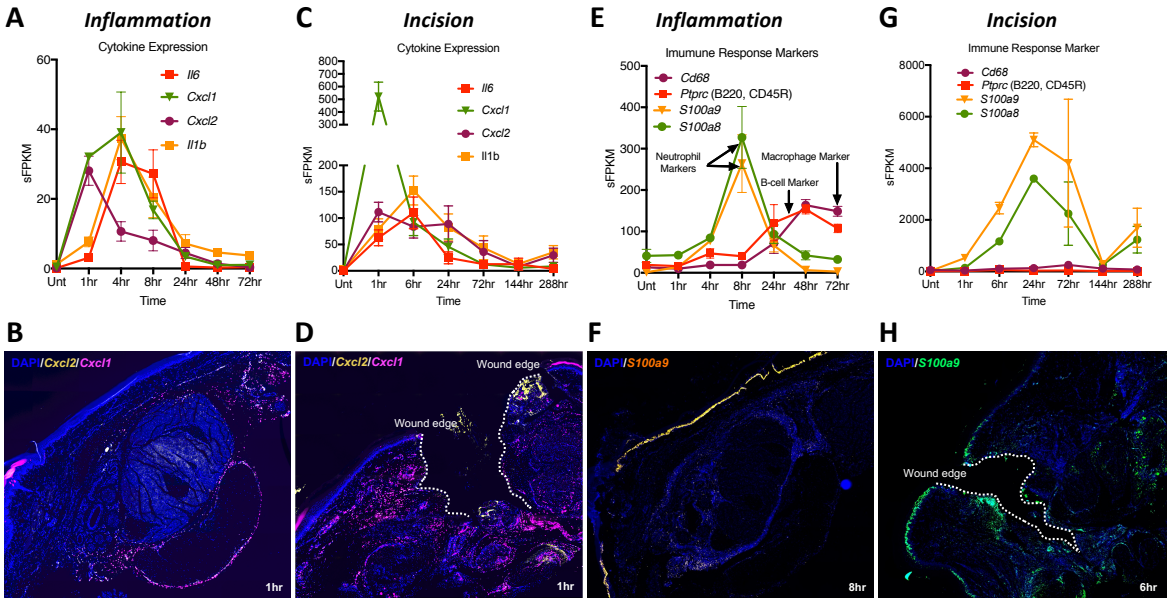

Figure 3 Gene expression patterns derived from RNA sequencing of peripheral inflammation and incision models. The expression levels of genes encoding secreted pro-inflammatory cytokines, *Il6*, *Cxcl1*, *Cxcl2*, and *Il1b* (A and C), as well as those related to immune response markers, *Cd68* (macrophage), *Ptprc* (B-cell), *S100a8* and *S100a9* (neutrophil) (E and G) were strongly differential between the inflammation and incision models. In addition, the peak of the expression patterns of these immune response genes were different. It varied between 1hr, 4-8hrs, and more sustained expression out to 72hrs (E and G). The gene expression patterns suggested that important types of cells have similar expression profiles in the two models, but in the incision model, expressions of many cytokines were greater compared to the inflammation model. The multiplex in situ hybridization images validated the localization of each significant gene (B, D, F, and H). We found that S100a9 was expressed not only in neutrophils but also in keratinocytes in both models (F and H).

was supported by the JSPS Overseas Research fellowship, we will conduct RNA-Seq from the human wound samples and confirm whether similar gene expression patterns will be observed from the animal models. Simultaneously, we will conduct primary culture experiments using rat cells obtained from dorsal root ganglion so that we can unravel mechanisms of wound pain and validate the biomarker candidates we identified from the animal models. All the research findings will be published in internationally important scientific journals, as appropriate.

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