Potential peripheral biomarkers for chronic pain

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Table of Contents

Table of Figures and Tables

Abstract

Chronic pain biomarkers can assist clinicians to diagnose patients, identify underlying mechanisms of disease, reduce the time and cost to reach a decision in early clinical trials and guide personalized pain treatments. Unfortunately, to date, there is no validated biomarker for chronic pain due to the difficulty in accessing the central nervous system. However, emerging literature has consistently provided evidence for the involvement of the immune system to play a substantial role in the modulation of chronic pain. Thus, this thesis examines components of the immune system such as Toll like receptor (TLR) signalling, peripheral immune cells and pro-inflammatory cytokine as an accessible source which may mirror similarities in brain immune cells and capture the changes in chronic pain state.

Therefore, the purpose of this thesis was to examine the use of peripheral immune cell reactivity as a potential biomarker for chronic pain. The first study was conducted in heterogeneous chronic pain and pain-free cohorts. Peripheral blood mononuclear cells (PBMCs) were isolated and stimulated with various TLR agonists to generate a pro-inflammatory cytokine interleukin-1β (IL-1β) concentration response curve. Chronic pain patients displayed significantly enhanced expression of IL-1β compared with the pain-free cohort hence the TLR responsiveness demonstrated face validity as a chronic pain biomarker.

The second study demonstrated the translatability of the importance of TLR responsiveness in a preclinical neuropathic pain model. IL-1β levels were quantified from basal and TLR2/4 agonist stimulated isolated rat PBMCs and spinal cord tissues, and together with the behaviour responses were used to generate statistical models. The main findings of this study were the inclusion of basal and TLR agonist stimulated outputs were required to predict the presence of pain and severity of allodynia with high sensitivity and specificity and that peripherally collected outputs correlated with the outputs from the spinal cord, suggesting the ability of peripheral outputs to give insight into

central signalling. In addition, a mathematical model developed from rat studies using peripheral and central tissues was able to identify chronic pain patients by their peripheral blood response with high accuracy.

The final study assessed TLR responsiveness of isolated PBMCs collected from a cohort of medication overuse headache (MOH) patients to determine the efficacy of a novel treatment for headache (ibudilast). This study consisted of MOH patients on 8 weeks of either placebo or ibudilast treatments. After 8 weeks of treatment, both groups did not experience a change in their headache frequency or intensity. However, a significant reduction in the TLR responsiveness occurred in the ibudilast group. These data provide the first evidence of a biomarker for ibudilast treatment.

In sum, this thesis provides evidence that peripheral cells are a good source to be biomarker for chronic pain and the importance to assess TLR signalling as an approach to capture the dysregulated immune system as a result of chronic pain. The discovered biomarkers require further replication and validation before than can be routinely used. However, the present finding can assist with the development of future cellular biomarkers for chronic pain.

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Kwok YH, Tuke J, Nicotra LL, Grace PG, Rolan PE, Hutchinson MR (2013) TLR 2 and 4 Responsiveness from Isolated Peripheral Blood Mononuclear Cells from Rats and Humans as Potential Chronic Pain Biomarkers. *PLoS ONE* 8(10), e77799.

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Abbreviations

ATP	Adensoine triphosphate
cAMP	Cyclic adenosine monophosphate
CCI	Chronic constriction injury
CCL	Chemokine (C-C) motif ligand
CNS	Central nervous system
COX	Cyclooxygenase
DAMPs	Danger-associated molecular patterns
DRG	Dorsal root ganglia
ELISA	Enzyme-linked immunosorbent assay
FCS	Fetal calf serum
FDA	Food and Drug Administration
fMRI	Functional magnetic resonance imaging
GABA	γ-aminobutyric acid
GFAP	Glial fibrillary acidic protein
KO	Knockout
IL	Interleukin
IκB-α	NFκB inhibitor α
iNOS	Nitric oxide synthase
IRAK	IL-1 receptor-associated kinase
LPS	Lipopolysaccharide
MAPK	Mitogen-activated protein kinase
MOH	Medication overuse headache
MyD88	Myeloid differentiation primary response gene 88
MSU crystals	Monosodium urate crystals

NGF	Nerve growth factor
NF-κB	Nuclear factor- κB
NMDA	N-methyl-D-aspartate glutamate
NNT	Number needed to treat
NO	Nitric oxide
Nox2	NADPH oxidase 2
NRS	Numeric rating scale
NSAIDS	Nonsteroidal anti-inflammatory drugs
P2X4	Purinergic receptor
PAMPs	Pathogen-associated molecular patterns
PBMCs	Peripheral blood mononuclear cells
SSRIs	Selective serotonin reuptake inhibitors
StEP	Standardized Evaluation of Pain
SDSN	Supernatant of damaged sensory neurons
TCA	Tricyclics antidepressants
TIR	Toll/IL-1 receptor
TGF- β1	Transforming growth factor beta
TLR	Toll-like receptor
TNF-α	Tumour necrosis factor alpha
TRAF6	Tumour necrosis factor receptor 6
TRIF	TIR-doman containing adapter-inducing interferon-β
VAS	Visual analogue scale
VRS	Verbal rating scale