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Pain's peptide signature

Michael Costigan

F.M. Kirby Neurobiology Center, Children's Hospital Boston, Harvard Medical School, 3 Blackfan Circle, CLS 12255, Boston, MA 02115, USA, Michael.Costigan@childrens.harvard.edu

Chronic pain, and especially persistent pain caused by an identifiable lesion to the nervous system (neuropathic pain), is a clinical concern of intense ongoing research. There is clearly a need for improved therapies. Multiple mechanisms have been discovered and illuminated within this field (reviewed in [1]) and medical research has in a few notable cases, such as gabapentin, resulted in tangible benefit [6]. Much work remains, however, in order that the mechanisms that underlie the generation of neuropathic pain can be elucidated and novel therapies developed rationally. Within the last few decades, genomic science has been added to the tool box of methods that can be used to define and understand this disease. Among these methods are cloning, transcript abundance quantification, genetic trait association, and genetic manipulation of model organisms. As technology improves, each of these methods aspires to be genome-wide, which will allow us, in theory, to peer at will into the innermost workings of an organism ([9] and references within this issue). These nucleic acid based methods have become so fashionable that it is rare to see a research article that does not contain them. Unfortunately, this focus on DNA and RNA often ignores the fact that a cell is made of protein and that the principle method of communication within the nervous system is electrical, i.e. via action potentials. Of course the body is the product of genes, proteins and physiology and each mechanism must be considered in context, which is why the advent of protein quantification *en masse* is such an exciting step forward.

Up until recently, protein quantification has been confined to Western blots, which use antibodies to assay proteins one by one. Indeed, early, and in some cases, current, attempts at mass protein analysis continued to use such technology. These methods are however unlikely to achieve the scalability required to become truly genome-wide, due to the unpredictable chemistry of differing antibody/epitope interactions. Quantitative peptide analysis by mass spectrometry, however now promises to fill this void, allowing us to potentially screen all of the proteins made by the cell in a single experiment. Most often this process separates the cellular protein in an initial chromatography step followed by high-resolution tandem mass spectrometry, dividing the proteins into individual peptides whose characteristic signatures can then be identified and quantified (reviewed in [2]).

An article by Oki et al within this issue [10] ushers in this exciting new chapter, as one of the first well controlled proteomic screens of human chronic pain tissue. Peripheral nerves with painful complex regional pain syndrome type 2 (CRPS-2) or painful neuroma and non-

Conflict of interest statement

I have no conflicts of interest.

chronic pain control nerves were screened in cadaver and post-operative patients. Each of the seven chronic neuropathic pain patients had suffered ongoing pain for at least 6 months that was refractory to several treatment protocols. When lidocaine was introduced into the nerve injury site it resulted in the disappearance or substantial attenuation of the pain, strongly suggesting the cause of the persistent pain within these patients was ongoing neuronal activity originating from the injured nerve. Because the authors had previously found nerve resection to be a valid treatment protocol in such cases [12], the dissected painful neuromas were available for analysis.

Using high-resolution tandem mass spectrometry, metallothionein (MT) was identified present in all control non-painful nerves but not found in any of the seven painful neuroma nerves. Next, this result was confirmed by Western blot and the site of MT expression within the non-painful nerves was determined to be Schwann cells by immunohistochemistry. MT is abundantly expressed in multiple tissues and is thought to act to regulate cellular copper and zinc distribution, protect against oxidative cell stress, alter gene transcription, detoxify heavy metals, and act in immune cell biology [11]. With so many postulated roles, it is difficult to determine what role it may play in regulating ongoing action potential generation within the painful neuroma. The authors speculate that MT may act through removal of reactive oxygen species within the Schwann cells of a healthy nerve, however such a mechanism remains to be proven. It is of course possible that lack of MT is not directly responsible for the resulting pain at all, but that it is just part of a more general protein dysregulation that occurs in otherwise injured Schwann cells, that cause persistent afferent discharge through other mechanisms [4]. Whether MT plays a direct role or not its complete lack of expression relative to high levels in all 'normal' nerves does suggest that this gene would make a strong biomarker for chronic pain initiated within a peripheral nerve. In addition, this finding does lend itself to investigating an explicit role for MT in normal nerve function versus neuroma pathophysiology.

Unbiased protein analysis of the nerve has the advantage of simultaneously assaying axonal proteins, nerve support cells and any immune cells present giving this method an unprecedented depth of field. However it should be noted that MT is a high abundance protein expressed heavily by healthy Schwann cells and many of the thousand or so peptide signatures identified by this study are from proteins that are expressed at relatively high levels. So providing the change in MT expression is shown to be mirrored at the transcriptional level, current mRNA profiling techniques (arrays and especially mRNA-seq [5]) should have readily identified this gene along with many more dysregulated targets [8]. If realized such extra data may have allowed a more systems wide analysis of Schwann cell biology between painful and non-painful nerves and therefore potentially greater biological insight. However mass spec analysis is making great advances toward true genome-wide coverage [3] and in addition mRNA expression most certainly does not always faithfully correspond to protein concentration [7]. So as peptide profiling methods develop we will be able to assay cellular mechanisms like never before, examining the true molecular composition of the cell, defining the composition of each sub-cellular component, and assaying state-specific phosphoproteins and other post-translational modifications. Whatever the medium assayed, DNA, RNA or protein, unbiased techniques are quite rightly becoming a mainstay of biological science, but such methods should only be considered the start of the

journey to determine biological mechanism. The study by Oki et al can be heralded as an exemplary early example of unbiased protein analysis and I am quite sure it will lead to many additional significant discoveries in pain research.

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