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Dr.sc.hum.

Genome-wide Identification and functional validation of microRNAs and mRNAs in chronic pain

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Chronic pain is a complex disorder that tremendously affects the quality of human life. Understanding the detailed molecular mechanisms in the establishment and maintenance of chronic pain is required owing to limitations in availability of efficacious analgesics and their significant side effects. In an attempt to identify new drug targets in chronic pain treatment, we employed a combination of high-throughput screening, molecular, *in silico* and behavioural approaches in clinically-relevant animal models. The current dissertation not only helps the researchers to understand mechanistic importance of several microRNAs and novel molecular targets, such as the chloride channel *Clcn3* and the hematopoietic growth factors, *GM-CSF and G-CSF*, but also helps to develop a comprehensive understanding on genome-wide trasncriptome changes occurring at microRNAs and mRNA levels in chronic pain conditions. This dissertation also opens up a whole plethora of potential therapeutic options in terms of many candidate microRNAs and mRNAs for further studies.

The thesis entails the first study to address the importance of microRNAs in the development and maintenance of tumor-mediated chronic pain. Via genome-wide microRNA screening, a subset of 57 miRNAs was identified in the dorsal root ganglia (DRG) of mice bearing bone metastases in the calcaneous bone of the hind paw. We performed detailed analyses on five un-annotated microRNAs sequences, which showed very strong expression in DRGs and were found to be deregulated in DRGs following tumor induction. To interfere with pathophysiological expression levels of microRNAs in DRGs *in vivo*, we established effective protocols for intrathecal delivery of microRNA inhibitors or mimics and demonstrated the efficacy of selective manipulations in miRNA expression *in vivo*. Our behavioral analysis in the bone-metastases model indicated that inhibition of the pathophysiological increase in expression of miR-1a-3p or miR-34c-5p in the DRGs markedly attenuated tumor-mediated hyperalgesia. Augmenting miR-370-3p in DRGs led to exaggerated tumor-mediated hyperalgesia. Furthermore, reversing pathophysiological decrease of miR-483-3p in tumor-bearing mice attenuated tumor-

mediated hyperalgesia. In contrast, inhibiting the pathophysiological increase in miRNA-544-3p expression or augmenting the pathophysiological decrease in miR-291b-5p in tumor-bearing mice did not elicit any impact on tumor-mediated hyperalgesia. All in all, we report a pronociceptive role for miR-1a-3p, miR-34c-5p and miR-370-3p, an anti-nociceptive role for miR-370-3p and a dispensable role for miR-544-3p or miR-291b-5p expression in the DRG in the modulation of tumor-mediated hyperalgesia. We also performed bioinformatics analysis on mRNA targets of selected miRNAs amongst the tumor-induced set and our detailed analyses identified Clcn3, a chloride channel, as a target of miR-1a-3p. We observed that Clcn3 is broadly expressed in DRG neurons and its expression is reciprocally regulated with respect to miR-1a-3p expression in the DRG following peripheral tumor induction. We observed that miRNA-1a-3p binds to 3'UTR of *Clcn3* and regulated its translation. Specifically knocking *Clcn3* expression down in sensory neurons *in vivo* resulted in exaggerated tumor-mediated hyperalgesia. Thus, we established miR-1a-3p-Clcn3 as a novel and functional promising miRNA-mRNA regulation pair in modulation of cancer pain.

Genome-wide transcriptome analysis via microarray screening revealed a very condensed list of 28 annotated and 7 un-annotated tumor-induced pain-related genes, which were deregulated in tumor conditions in DRGs. Out of these, mechanistic aspects are known for only few genes in the context of chronic pain to date, suggesting a large potential for future studies in addressing novel potential mediators of tumor-mediated pain.

In previous experiments, we have identified hematopoietic factors, GM-CSF or G-CSF, as key mediators of tumor-nerve interactions and cancer pain. We observed that knocking-down the GMCSF receptor specifically in the sensory neurons attenuated tumor-mediated hyperalgesia without affecting the tumor growth. Genome-wide mRNA expression profile revealed that exposure to GMCSF regulates the expression of a broad variety of transcripts, including chemokines, ion channels, transcription factors, proteases and predominantly STAT-3 targets, in peripheral sensory neurons of the DRG, thereby helping understand the molecular mechanisms underlying the pronociceptive role of these hematopoietic factors.

In neuropathic pain conditions, next generation deep sequencing revealed a subset of microRNAs which are differentially regulated in DRGs of rats which are genetically predisposed to either develop or protected from neuropathy-induced pain behavior. Along the same lines,

genome-wide transcriptome analysis via microarray expression arrays revealed a subset of mRNAs differentially regulated in the DRGs of these two lines of neuropathic rats. These results suggest there is indeed an involvement of molecular machinery at different hierarchical levels (i.e. microRNA, mRNA, mediators) involved in determining the genetic predisposition to chronic nociceptive insults. Further mechanistic analyses to investigate orchestrated regulation of different microRNAs and their target genes in these two animal lines are in order for future studies directed towards understanding neuropathic pain.

The results of this dissertation work thus deliver valuable new insights into modulation of cancer pain and neuropathic pain by genetic regulation at the level of miRNAs and mRNAs and yield attractive, novel targets which show therapeutic potential.