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MEETING ABSTRACT

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Successful rescue of impaired fear extinction leads to dynamic regulation of microRNAs in the amygdala

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Background: Current treatments to overcome excessive fear in anxiety disorders, including exposure-based therapy, are far from being optimal. A basic principle of exposure-based therapy is the formation of long-lasting fear-inhibitory memories, which depends on *de novo* gene transcription of plasticity-related genes, amongst others. Recent evidence suggests that gene transcription after a learning event is influenced by non-protein-coding RNAs (ncRNAs) including microRNAs (miRNAs), for example, miR-128b which has been demonstrated to be involved in the formation of fear-inhibitory memory. However the role played by miRNAs promoting enduring fear-inhibitory memories in individuals with extinction deficits remains to be determined.
Methods: Mimicking anxiety patients, 129S1/SvImJ (S1) mice exhibit profound resistance to induce fear extinction. We have shown that persistent context-independent extinction in S1 mice can be induced using dietary zinc restriction. Utilizing dietary zinc restriction as a tool, we can elucidate the underlying mechanisms leading to the successful rescue of impaired fear extinction. To examine to role of miRNAs in this process we used microarray technology and subsequent RT-PCR to assess the regulation of miRNAs in the amygdala of S1 mice 2 h after the successful rescue of impaired fear extinction. Anatomical localization of candidate miRNAs was demonstrated by fluorescent *in situ* hybridization.
Results: Microarray analysis and consecutive RT-PCR confirmation revealed an altered regulation in a select set of miRNAs, including an increase in miR-144 expression in the amygdala of S1 mice. Genes regulated by miR-144 remain to be elucidated; however we show regulation of the predicted target retinoic acid receptor beta (RXRβ), a plasticity-related gene, in S1 mice during rescue of impaired extinction. Experiments establishing the link between miR-144 and fear-inhibitory memories are ongoing. To examine the anatomical localization of candidate miRNAs in a neuronal subtype-specific manner we have set up and validated a fluorescent *in situ* hybridization. Initial results on candidate miR-144 demonstrate a highly selective expression restricted to the central amygdala.
Discussion: In conclusion, rescuing impaired fear extinction in a persistent and context-independent manner is associated with dynamic regulation of the expression of select miRNAs, which can interact with protein-coding synaptic plasticity genes. One candidate, miR-144, exhibits a highly selective expression restricted to the central amygdala, and we have confirmed the downregulation of RXRβ, a predicted target of miR-144. Understanding the role of miRNAs in the rescue of impaired fear extinction may shed light on novel therapeutic targets to treating these debilitating disorders.
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