Transcriptomics Signature of Type I Narcolepsy (T1N)

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Introduction

Narcolepsy (type 1) is a rare and severe sleep disorder characterized with the exclusive and extensive destruction of orexin-producing hypothalamic neurons [1]. The patients exhibit cataplexy (episodes of partial or complete paralysis of the voluntary muscles), sleep paralysis, hypnagogic hallucinations, as well as excessive daytime sleepiness and fragmented nocturnal sleep. Orexin-A and -B are small peptide neurotransmitters produced only by a cluster of neurons in the lateral hypothalamus which stimulate target neurons that promote wakefulness, regulate appetite and conserve energy. The mechanisms involved in the selective destruction of orexin neurons are not yet elucidated but its association with certain environmental triggers (vaccine against flu virus - Pandemrix), genetic susceptibility (strong association with the human leukocyte antigen – HLA), T-cell receptor (TCR) and other immune loci (such as CTSH, P2RY11, ZNF365, IFNAR1, TNFSF4) implicate an immunopathological process [2].

Experimental Design

To investigate whether an (auto)immune process could lead to narcolepsy development and to decipher the mechanisms involved in the selective loss of orexin neurons, we've developed a mouse model of immune-mediated narcolepsy (Orex-HA) to determine the molecular profiles of the hypothalamus infiltrating T-cells [3]. The Orex-HA mice are genetically engineered to express hemagglutinin (HA) of the H1N1 influenza virus as a self-antigen selectively on orexin neurons. In this experimental model, naive HA-specific CD4 and CD8 T-cells were injected into the mouse at day 0 and activated via immunization with the flu vaccine (Pandemrix) the following day. Vaccination results in the activation of HA-specific CD4 and CD8 T-cells, which then migrate into the hypothalamus and target the orexin neurons expressing HA. At the peak of CNS infiltration (day 14), the mice were euthanized and the host (CD45.1-) and donor (CD45.1+) CD4 (CD44+CD62L-) and CD8 (CD44+CD62L-) T-cells were cell sorted (FACS) from the hypothalamus, spleen and cervical lymph nodes (cLNs). Total RNA was then isolated and sent for mRNA sequencing to better understand the transcriptomics signature of narcolepsy.

Results

Since the focus of this objective was to determine the molecular profiles of the hypothalamus-infiltrating T-cells, we combined the spleen and cLNs samples into "periphery" and limited the analysis to "Brain vs Periphery" for each of the T-cell populations. We've interrogated the differentially expressed genes derived from the comparison of the brain versus periphery for each of the four different cell types separately. These gene lists were evaluated via Ingenuity Pathway Analysis software (IPA) for canonical pathway analysis, KEGG pathway analysis (Webgestalt), and gene ontology (GO) analysis (Webgestalt). After reviewing the top 10 pathways, a concise gene list was selected based on immunological relevance and curiosity. This gene list will be prioritized for the first level of validation via QPCR.

References

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