Regional differences in gene expression and promoter usage in aged human brains

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Research question and background

The brain is the most complex organ of the human body. It can be divided into different functional and anatomical regions established during development and maintained throughout life. The mechanisms that regulate normal brain function and differentiation are controlled by both genetic and epigenetic factors and alterations in these mechanisms can lead to neurodegenerative diseases. Although the tremendous advances in our understanding of the molecular mechanisms involved in brain function, less is known about the genetic mechanisms that are responsible for establishing and maintaining these differences throughout development, adulthood and aging. Insights into these mechanisms are required to understand the differential susceptibility of distinct brain regions to neuronal insults. In order to dissect the genetic mechanisms controlling brain functions we used Cap Analysis of Gene Expression (CAGE) to comprehensively profile the transcription start sites (TSSs) and promoter regions of the brain.

Methods and tissues used

RNA was isolated from caudate nucleus, putamen, frontal and temporal cortex and hippocampus from seven elderly individuals with no clinical signs of neurodegenerative disorders. RNA quality per tissue was assessed using the RNA Integrity Number (RIN). CAGE libraries were prepared according to the protocol described by Takahashi et al 2012 and sequenced on an Illumina platform.
DNA isolation and purification to detect methylation was carried-out following standard protocols. Genome-wide amplified input and output samples were hybridized to DNA Methylation 2.1M Deluxe Promoter Arrays.

Results and conclusion

We sequenced over 71million CAGE-tags corresponding to 70.202 promoter regions and 16.888 genes. Over 7.000 transcripts were differentially expressed, mainly due to differential alternative promoter-usage. Unexpectedly, 7% of differentially expressed genes were neurodevelopmental transcription factors. Functional pathway analysis on the differentially expressed genes revealed an overrepresentation of several pathways like Fibroblast-Growth-Factor, Wnt signaling and mGLURI pathway in hippocampus and striatum. The lasts two pathways have been implicated in neuronal damage.
We also found that although 73% of methylation signals mapped within genes, the influence of methylation on the expression-profile was small.
Despite some pathological findings consistent with aging, none of the seven donors used for this study showed any overt Alzheimer and Parkison's disease pathology. Nevertheless our study shows that genetic signatures related to neurodegeneration were already present in brain regions that are highly vulnerable to neurological disorders.

References:

1) Takahashi H, Lassmann T, Murata M**,** Carninci P. 5'end-centered expression profiling using cap-analysis gene expression and next generation-sequencing. Nat Protoc..2012 23;7(3):542-61