Analysis of age-related changes in gene expression in human microglia

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Research question and background

In this project we investigate the functionality and genetic program of microglia aging. In addition to the high quality fresh post mortem samples from NBB we receive samples from other European institutes and gradually we are assembling a useful collection of samples. We compare the gene expression profile and functionality of microglia isolated from human samples with microglia isolated from various strains of aged mice and transgenically senescence accelerated mice. The goal of this project is to provide a model description of aging of the neuroimmune system and its relation to neurodegenerative diseases.

Methods and tissues used

We have developed and optimized a robust protocol for the acute isolation of human microglia from autopsy brain samples (Olah et al., 2012). We have used parietal cortex samples from control patients (no neurodegenerative disease) and our goal is to isolate microglial cells from young (10-20 years), middle aged (50-60 years), and aged (80 years and older) donors and compare these samples using an RNA sequencing procedure.

This application requires the purest form of microglia samples and accordingly, an extra purification step has been added to the already published protocol.

With the use of a FACS sorter, we are able to isolate CD11b and CD45 positive cells and these double-labeled cells are collected, resulting in pure microglia samples from which we isolate RNA.

For a reliable comparison between young and old microglia we need at least 8 samples in each group with sufficient mRNA yield of high quality.

Results and conclusion

Tissues 2011

In 2011 we obtained 11 samples with the following specifications:

age: 49-89

pH CSF: 6.02-6.88

Three of these samples have been used to optimize the isolation protocol and finish a method paper, describing the isolation protocol (Olah et al., 2012).

From the remaining eight samples, RNA was isolated, cDNA was transcribed and these samples were used for real time PCR experiments for a manuscript in preparation (Raj et al.,)

Tissues 2012

In 2012 we have obtained 6 samples with the following specifications:

age: 62-102

pH CSF: ≥ 6.5

Based on our experience on RNA isolation from acutely isolated microglia of the previous year we decided set a restriction on the pH value of the post mortem CSF, which should not be less than 6.5. Furthermore, after the RNA isolation, the quality of the RNA was checked on a Bioanalyzer. For our purpose, the RIN values of the RNA samples should be between 7.0 and 10. Samples with a RIN value below 7.0 have been

omitted. As observed previously, low pH values of the CSF correlated with low RIN values and we decided to persist in the criterium criterion of a pH value of 6.5 or higher.

Total number of samples obtained in 2011-2012

NBB no.	datum obductie	sex	age	Klinische diagn.	pH csf	pmd
2011-044	2011-05-09	m	51	CONTR	7,05	07:45
2011-045	2011-05-18	f	68	AD!	6,02	03:30
2011-046	2011-05-18	f	89	CONTR	6,67	04:45
2011-069	2011-08-24	m	49	OTHER	6,23	06:15
2011-073	2011-09-05	m	89	AD!	6,66	04:56
2011-081	2011-09-28	m	55	CONTR	6,88	07:30
2011-082	2011-10-01	f	84	CONTR	6,1	05:55
2011-090	2011-10-11	f	85	DYSTO	6,51	08:25
2011-096	2011-10-26	f	70	CONTR	6,55	06:15
2011-104	2011-11-07	m	70	Controle / cogn. Imp.	6,4	06:20
2011-114	2011-11-29	f	81	CONTR	6,77	05:30
2012-001	2012-01-04	f	89	CONTR	6,75	5:40
2012-006	2012-01-30	m	62	PIFTD	6,71	4:40
2012-048	2012-05-10	m	81	DEPRI	6,7	6:40
2012-067	2012-07-08	m	102	CONTR	6,64	5:00
2012-082	2012-08-09	f	101	CONTR	6,57	5:10
2012-112	2012-10-25	m	85	CONTR	6,54	7:25

Reference

Olah M, Raj D, Brouwer N, De Haas AH, Eggen BJ, Den Dunnen WF, Biber KP, Boddeke HW. An optimized protocol for the acute isolation of human microglia from autopsy brain samples. Glia (2012) 60 :96-111.