Expression Profiling of Microglia and Macrophages Using a Novel Lipidomic TaqMan® Array on Cards and Plates

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INTRODUCTION
Cellular lipidomics is the analysis of metabolism, transport, and localization of lipids within cells (1). The identification and quantification of lipids, and biochemical analysis of lipid metabolism are integral parts of this concept. This rapid advancement of lipid species profiling has been driven by a substantial progress in chromatography and mass spectrometry (2). In addition to this prevailing view of lipidomics, mRNA expression analysis of lipid-related genes and definition of their regulatory networks still remain to be developed for a holistic view of lipidomics. DNA-microarrays and the development of high-throughput real-time qPCR systems have enabled researchers to perform a comprehensive transcriptional analysis of all lipid-related genes. DNA-microarrays offer the complete genomes from various species, including well-annotated human and mouse genes. However, in any given cell type, e.g., microglia cells or macrophages, only a fraction of all lipid-related genes is expressed and regulated at the mRNA level. Moreover, DNA-microarrays have a lower dynamic range and are less precise and sensitive than quantitative real-time qRT-PCR.

Our aim was to use the Applied Biosystems TaqMan® Array technology (3) for the quantitative analysis of informative lipid-regulated genes in microglia and macrophages. Of particular interest was to study the dynamic gene expression under conditions mimicking sterile overload, inflammation, and/or Marty acid stimulation.

TAQMAN ARRAY WORKFLOW

RESULTS
Figure 1 displays mean RQ values of stimulated versus untreated cells using RQ Manager calculation with Gapdh as reference gene. Figure 2 shows a Real-time StepMaster analysis after applying the NormFinder algorithm and Benjamini-Hochberg adjustment of the False discovery-rate at p<0.05. The results obtained with the Lipidomic TaqMan Array Cards were reproduced and confirmed using TaqMan Array Plates.

PHAGOCYTES AS TARGETS FOR EXPRESSION PROFILING
Phagocytes connect immune response with lipid metabolism. Microglia, BV-2 cells, and bone marrow-derived macrophages were stimulated for 24 h with 10 μM of the LXr and RXR agonists T0001317 and 9-cis retinoic acid (RA) to mimic sterile overload, 20 ng/ml lipopolysaccharide (LPS) and 50 ng/ml interferon-gamma (IFN-γ) for pro-inflammatory stress, and 20 ng/ml LPS + 100 μM docosahexaenoic acid (DHA) as anti-inflammatory lipid agonist. This approach enabled the cross-wise comparison of related cell types and a dynamic view on gene expression modulated by bioactive compounds.

LIPIDOMIC TAQMAN ARRAY CONFIGURATIONS
The Lipidomic TaqMan® Array Card is based on the 384-well microfluidic card format 48, designed for use with the Applied Biosystems 7900HT Fast Real-Time PCR System (http://www.appliedbiosystems.com/). The 2μL reactions decrease sample consumption and the pre-loaded primers and probes reduce pipetting steps. TaqMan® Array Plates are 96-well microtiter plates that have the same 48 assays. TaqMan® Assays dried down in every well position. Although the reaction volume is larger (2μl/well), plates can be used on more Realtime PCR instruments.

RUNNING TAQMAN ARRAY CARDS AND PLATES
Total RNA was isolated and checked with Nanodrop 100 / Agilent 2100 bioanalyzer. First strand cDNA synthesis was carried out and 100 ng of cDNA RNA-equivalent) were loaded together with 2x TaqMan Gene Expression Master Mix in a total of 10μl per reaction. The TaqMan Array Cards were spun and sealed before loading into a 7900HT Fast Real-Time PCR System. Relative quantification runs were performed with default thermal cycling conditions. The same reactions were analyzed on TaqMan Array Plates using universal cycling conditions on a 7900HT Fast Real-time PCR system. Data analysis was performed with RQ Manager and Real-time StepMaster.

CONCLUSIONS
• The Lipidomic TaqMan® Array Cards and Plates are optimized tools for high-throughput quantitative transcript analysis of the most dynamic lipid-related genes in phagocytes and many other cell types.
• Its combination with the Real-time StepMaster further enables researchers to generate statistically evaluated high-quality data.
• This technology will be very useful for transcriptomics as part of the rapidly progressing field of lipidomics.

REFERENCES

Figure 1: Relative mRNA expression values in three myeloid cell types
(A) Stimulation with T0001317 + 9-cis RA and drug-induced replenishment on several genes of lipid metabolism (B) the pro-inflammatory stimuli LPS and IFNγ strongly repressed most lipid-related genes, and (C) co-incubation with DHA attenuated the repressing effect of LPS.

Figure 2: Log 2 RQ values of myeloid cells.
Upper part: non-significant changes and lower part: significant changes in duplicate measurements of two biological replicates. P<0.05.