Single cell microRNA and mRNA profiling reveals unique gene expression signatures and heterogeneities in mouse ES cells

Caifu Chen, Ruoying Tan, Dana Ridzon, Leila Bahreinifar, Linda Wong, Karl Guegler, R&D, Applied Biosystems, Foster City, CA 94404;
William M. Strauss, Department of Molecular, Cellular, & Developmental Biology, University of Colorado, Boulder, CO 80309, USA.

ABSTRACT

We describe a new method for simultaneously quantifying 237 mouse microRNAs (miRNAs) and 21 mRNAs from single cells including embryonic stem (ES) cells, embryoid bodies (EBs), 3T3 cells, or splenocytes. The method is based on multiplex RT, multiplex preamplification, and singleplex real-time TaqMan® PCR assays. Single cell expression signatures could classify individual ES, EBs, or somatic cells. Significant inter-cell variations of both miRNA and mRNA expression within ES cell lines indicated the heterogeneity of ES cells. The highest variability was observed among EB cells, demonstrating that EBs undergo differentiation at different stages. Interestingly, ES marker gene Oct4 and signaling gene Tdgf1 were highly co-expressed. Both were absent in 3T3 and splenocyte cells, highly expressed in ES cells, and significantly reduced in EB cells. Results indicated that Oct4 and Tdgf1 might be co-regulated during ES differentiation. The total number of expressed miRNA genes in ES, EB, and somatic cells remained constant. However, their expression levels were significantly elevated during differentiation, further suggesting the involvement of miRNAs in cellular development and specification. Furthermore, there was no correlation in the expression levels between miRNAs and their predicted target mRNAs, thereby supporting a translational repression model.

INTRODUCTION

MicroRNAs are endogenous RNAs of ~22 nucleotides that play important regulatory roles in animals & plants. We propose that miRNAs are likely important regulators for stem cell self-renewal. This is based on the observation that distinct sets of miRNAs are specifically expressed in pluripotent ES cells but not in differentiated EBs (1), and that EBs express miRNAs not found in ES cells (2). We utilized newly developed Multiplex PreAmp TaqMan® microRNA assays to profile 237 miRNAs & 21 mRNAs. The objective is to determine single cell expression signatures of miRNAs and mRNAs in ES and differentiated cells. Single cell expression analysis could be useful in determining cell identity and monitoring the ES differentiation.

MATERIALS AND METHODS

No. genes analyzed: A total of 237 mouse miRNAs and 21 stem cell-related mRNAs were used in this study.
RNA samples: Seventy individual cells were randomly chosen from mouse splenocytes, 3T3, ES cell line and its differentiated embryoid bodies at day 3. Aggregated ES cells were cultured under feeder- and serum-free condition (Kanno et al. 2004). Differentiated EB cells were harvested at day 3 after plating ES cells in an untreated Petri plate with culture media. Single cells were manually picked after trypsin treatment. Cells were re-examined under microscope to confirm one cell per well.
Multiplex PreAmpTaqMan® microRNA assays: The assay includes three steps, multiplex RT, preamplification, and single-plex TaqMan® PCR. Single cell was lysed by heating at 95°C for 5 min before performing RT reaction. Real-time PCR was performed on an AB 7900HT Sequence Detection System.
Data analysis: Averaged C_T of three constantly expressed miRNAs (miR-19a, -19b, and -20) was used to confirm the presence of single cell per reaction. All miRNAs with C_T >37 were assumed not expressed. The copy number per cell was estimated based on the C_T value and a standard curve of spiking synthetic lin-4 miRNA. Agglomerative hierarchical clustering was performed using CLUSTER program (3).

RESULTS

Fig. 1. Schematic description of Multiplex PreAmp TaqMan® microRNA and mRNA assays from single cells

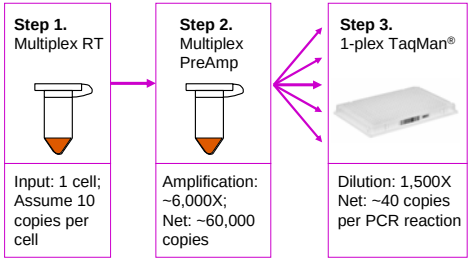


Table 1. Summary of miRNA expression in ES, EB, and 3T3 cells. MiRNA expression increased as differentiated. Highest variability in expression was observed between EB cells.

		ES	EB	3T3	Splenocytes
No. cells analyzed		24	22	8	8
Ave. copy No./cell		210	300	1270	960
% of miRNAs with	0 copy/cell	48	49	49	51
	>0, ≤10 copies/cell	15	11	9	8
	>10, ≤100 copies/cell	19	20	13	21
	>100, ≤1,000 copies/cell	14	16	15	12
	>1,000 copies/cell	5	4	14	8
Inter-cell CV (%)		18	25	17	24

Fig. 3. Single cell expression profile of 237 microRNAs and 21 mRNAs from a total of 70 individual ES, EB, splenocyte, and 3T3 cells

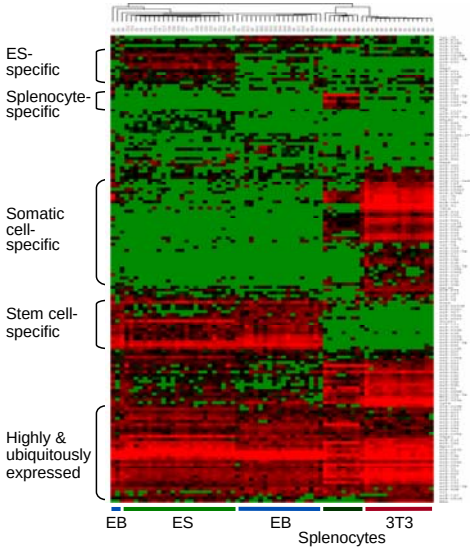


Fig. 2. Multiplex PreAmp miRNA assays are quantitative: Linear response of C_T to RNA input from 0.1X to 100X

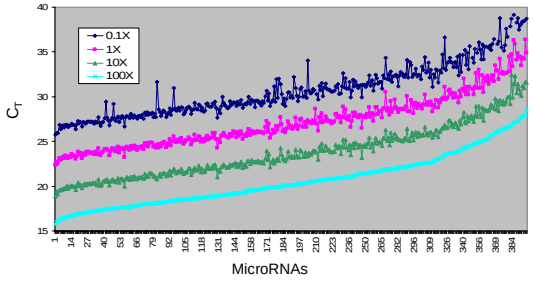


Fig. 4. Single cell profiling revealed that both OCT4 and Tdgf1 expression is highly variable among ES cells and reduced or absent in differentiated cells

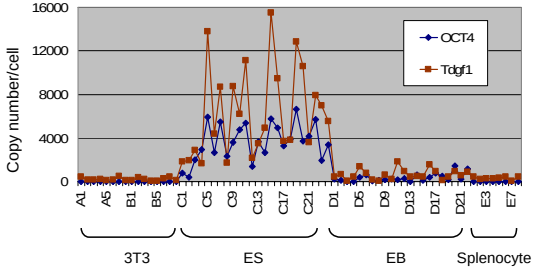
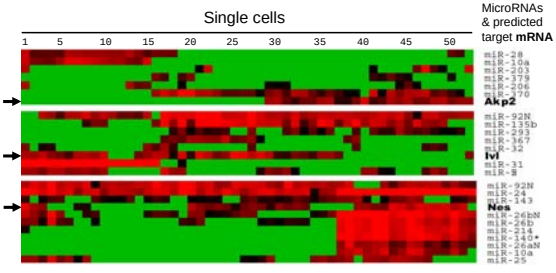


Fig. 5. No correlation of gene expression in ES, EB, and 3T3 cells between miRNAs and their punitive target mRNAs at the single cell level



REFERENCES

1. Houbaviy et al. 2003. Developmental Cell 5:351
2. Strauss et al. 2006. Mamm Genome: 17:833
3. Eisen et al. 1998. PNAS 95:14863-14868

TRADEMARKS/LICENSING

127PR18-01