Abstract
MEFV is a human gene that codes for an inflammation-related protein pyrin, pyrin regulates inflammation by directing the migration of leukocytes. It also interacts with other inflammation and apoptosis regulators. Mutation of MEFV leads to malfunctional pyrin and causes familial mediterranean fever — an auto inflammatory disease. This project aims to investigate the expression pattern of MEFV in cells under different states and provide a first-step understanding in the regulation process of MEFV expression. Here we demonstrated that the MEFV expression was down-regulated in response to cell differentiation in both RNA and protein level. Additionally, we discovered that both differentiated and undifferentiated cells expressed MEFV transcripts lacking exon 2.

Introduction
MEFV is a protein-coding gene that located on the short (p) arm of chromosome 16. When activated, it encodes an inflammation-related protein called pyrin. Pyrin is mainly expressed in certain leukocytes such as neutrophils, eosinophils, monocytes and dendritic cells. Inside these cells, pyrin conducts its function in inflammation control and infection response by interacting with cytoskeleton and targeting the immune cells to infection sites, it also controls the
inflammation response by slowing down leukocytes’ migration. Pyrin also plays its role by interacting with several regulators of apoptosis and inflammation such as ASC, p65, PSTPIP-1, and caspase-1.(1)

Mutated MEFV results in loss of function of pyrin, which causes Familial Mediterranean Fever (FMF). FMF is one of the most common autoinflammatory disease. It is estimated that as many as 1 in 5 people of Jewish, Armenian, Arab and Turkish heritage have one mutant copy of MEFV, and 1 in 200 people from these ethnic groups may have FMF. More than 80 mutations across the MEFV gene were identified to be FMF-related.(2)

These patients suffer from periodic fever, pain in abdomen and joints, arthritis, amyloidosis and many other symptoms as well as complications.

The purpose of this project was to determine how MEFV is expressed in undifferentiated pro myeloid cells and in pro myeloid cells induced to differentiate. Promyeloid cells are the precursors to many cell lines including cell lines in which MEFV is expressed.

Materials and methods

Cell culture & treatment: Human promyelocytic leukemia (HL-60) cells were treated with 10 nM Vitamin D3 and 50 nM tetradecanoyl phorbol acetate (TPA) to induce differentiation.

Immunoblotting: Purified HL-60 protein samples were separated by SDS-PAGE and transferred to a nitrocellulose membrane(Novex) which was then probed with mouse anti-MEFV monoclonal antibody 2C1(1:1000,Sigma) and anti-mouse Antibody (1:7500, Promega).

RT-PCR: HL-60 RNA samples were subjected to the reverse transcription PCR using 4 pairs of primers spanning the entire transcript.

Primers used in RT-PCR:

For amplifying region from exon 1 to exon 5:
- exon1F:TCTGCTGGTCACCTACTATG
- exon 5R:CTGCAGAAGTTCCCATTCTG

For amplifying region from exon 3 to exon 5:
- exon 3F:GACTCCCATGAAAGGAAGAG
- exon 5R:CTGCAGAAGTTCCCATTCTG

For amplifying region from exon 3 to exon 6:
- exon 3F:GACTCCCATGAAAGGAAGAG
- exon 6R: GTGCAAGATGTCTCCAATGTC

For amplifying region from exon 5 to exon 10:
- exon 5F:TGCTCGATGCGCTGATTG
- exon10R:CCTGCTTATGGATGTCTTGC

RT-PCR was performed using QIAGEN® One-Step RT-PCR Kit following instructions. 10ng of RNA was amplified in 10ul RT-PCR reaction. Temperature cycles: one cycle of 50°C for 30min and 95°C for 15min, 94°C for 30 s, 58°C for 30s, 72°C for 30s, and a final extension of 72°C for 5 min followed by a final hold at 4°C. Cycle number was 40.
2 μl of loading dye was added to each RT-PCR product. 5 μl of each product was then added to a 1% agarose gel, and electrophoresis was performed at 160 V. Band intensities were visualized by ethidium bromide in a UV trans-illuminator (Carestream). 100 bp DNA ladder (Trackit) was used. The PCR products were extracted by QIAquick PCR purification Kit (QIAGEN) following the manufacturer's instructions and subsequently sequenced by GENEWIZ®.

Results

**MEFV protein expression pattern is changed in response to cell differentiation.** We examined the MEFV protein expression by conducting the western blotting analysis. Results shown in Figure 1 indicates that a peptide of which size is about 51kDa is present in undifferentiated cells but not found in differentiated cells. This size corresponds to a MEFV isoform encoded by an alternatively spliced transcript lacking exon 2.

The full-length MEFV transcript encodes a protein approximately 86kDa in size, but it is unclear as to whether this isoform is present in either the undifferentiated or differentiated cells.

**Cell differentiation induces changes in production of MEFV transcript.** 4 pairs of primers were used in RT-PCR to identify MEFV transcripts (Figure 2). For primers pair 3F-5R and 3F-6R, bands of expected size appeared only in undifferentiated cells, no RNA fragment is picked up by these primers in differentiated cells (a, b). Primers pairs 1F-5R and 5F-10R successfully amplified RNA in both cell types, but with a reduced level observed in differentiated cell (c,d). While expression of GADPH, a housekeeping gene, was similar in both cell types.

Another observation that brought our interest was that when using primers pair 1F-5R to amplify PCR product, instead of the 1423bp fragment we expected in full-length MEFV, we found a shorter fragment with a size at about 790bp in both cell types (d). This is most likely to happen if exon 2 is spliced out (Figure 3). We sent out the product for DNA sequencing and the results verified that exon 2 was absent in both cell types.

![Figure 1 Western blotting analysis of MEFV](image)
Discussions

We have demonstrated in this project that MEFV gene expression is altered during cell differentiation. Expression of certain peptides are largely reduced in differentiated cell. What's more, the change in RNA transcript of MEFV is been observed. Taken together datas from immunoblotting and RT-PCR, the overall expression level of MEFV is reduced in differentiated cell.

Additionally, we discovered that exon 2 is spliced out in both cell types. In response to differentiation, cells down-regulate the expression of a 51kDa protein, which is the expressed...
size of the protein encoded by the exon 2 lacking transcript. Further Western blots with a different MEFV antibody would be required to confirm that this protein is indeed pyrin.
Exon 2 encodes for the bZIP transcription factor basic domain in pyrin protein, which is believed to interact with p65 and IκB-α, and in turns controls the activity of NF-κB and the expression of inflammatory genes. (3)
Questions remained for future study include how this shortened protein function in response to inflammation as compared with full-length pyrin, and what could we interpret from this when it comes to the pathology of FMF, since many disease-related mutations of MEFV happen in this domain.

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References