Q-PCR Analysis of GATA-1 in Umbilical Cord Blood: Optimizing RNA Extraction and Reverse Transcription Techniques

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Abstract

Rationale: Cord blood GATA-1 mRNA has been shown to serve as a biomarker of neoplastic hematopoiesis, but yields are often low and the assay challenging to optimize. In this study, we compared three RNA extraction protocols followed by three reverse-transcription assays to optimize our experimental yield.

Methods: Umbilical cord blood non-adherent MNCs (NAMNCs) were stimulated with Interleukin-1 (IL-1) to promote neoplastic hematopoiesis. NAMNCs were collected at 0, 24, 48 and 72 hours post-stimulation and RNA was isolated using the RNAqueous Plus Mini kit (Ambion) and Total RNA extraction kit (Pregenex). Samples were reverse transcribed using three kits: Quantitect Reverse Transcription Kit (Qiagen), AffinityScript qPCR DNA synthesis kit (Agilent), and High Capacity RNA-to-cDNA Kit (Applied Biosystems). The samples were also reverse transcribed using cDNA (Qiagen) and three qPCR supermixes were tested: TaqFast Supermix (Biorad), 2X Taq Supermix (Qiagen), and T&Q Probe Supermix (Biorad). The RNA expression of GATA1 was measured using qPCR (Biorad).

Results: We found that the standard curve efficiencies were optimal for Qo/Qp, Q/p, AffinityScript and Pregenex. Pregenex/AffinityScript and TaqFast demonstrated suboptimal efficiencies. In addition, there were no differences in efficiency using various qPCR supermixes.

Conclusions: The combination of Qo/Qp and Pregenex/Biorad was the most reproducible and thus more efficient. In addition, the TaqFast probe mix appears to be more versatile in the amplification of the GATA1 gene under various conditions.

Preclinical Results

Methods

Concentrate was given by expectant mothers and the umbilical cord blood was collected after delivery. The cord blood samples were processed immediately after their collection. All samples were dispensed of red blood cells via gravity to isolate MNCs and to prevent hypothermia. All samples were then thawed, divided of adherent cells and reseeded at 2-3 million cells per condition. Non-adherent RNA (NAMNCs) were then stimulated with recombinant human Interleukin-1 (IL-1) to promote neoplastic hematopoiesis. NAMNCs were collected at 0, 24, 48 and 72 hours post-stimulation and RNA was isolated using the RNAqueous Plus Mini kit (Qiagen) and Total RNA extraction kit (Pregenex). The samples were then reverse transcribed using three kits: Quantitect Reverse Transcription Kit (Qiagen), AffinityScript qPCR DNA synthesis kit (Agilent), and High Capacity RNA-to-cDNA Kit (Applied Biosystems). The samples were also reverse transcribed using cDNA (Qiagen) and three qPCR supermixes were tested: TaqFast Supermix (Biorad), 2X Taq Supermix (Qiagen), and T&Q Probe Supermix (Biorad). The RNA expression of GATA1 was measured using qPCR (Biorad) and 2X Taq Supermix (Biorad).

Discussion

In this study, we found there was a higher RNA yield when using the Pregenex extraction kit on NAMNCs compared to the Quantitect extraction kit. However, this difference in yield was quite variable from sample to sample. All samples were dispensed of red blood cells via gravity to isolate MNCs and to prevent hypothermia. All samples were then thawed, divided of adherent cells and reseeded at 2-3 million cells per condition. Non-adherent RNA (NAMNCs) were then stimulated with recombinant human Interleukin-1 (IL-1) to promote neoplastic hematopoiesis. NAMNCs were collected at 0, 24, 48 and 72 hours post-stimulation and RNA was isolated using the RNAqueous Plus Mini kit (Qiagen) and Total RNA extraction kit (Pregenex). The samples were then reverse transcribed using three kits: Quantitect Reverse Transcription Kit (Qiagen), AffinityScript qPCR DNA synthesis kit (Agilent), and High Capacity RNA-to-cDNA Kit (Applied Biosystems). The samples were also reverse transcribed using cDNA (Qiagen) and three qPCR supermixes were tested: TaqFast Supermix (Biorad), 2X Taq Supermix (Qiagen), and T&Q Probe Supermix (Biorad). The RNA expression of GATA1 was measured using qPCR (Biorad) and 2X Taq Supermix (Biorad).

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