Rapid Detection of the Apoptosis Related Genes (BCL-2, BAD, BAX) in 12 Hours 1,25 $(OH)_2D_3$ Treated HL-60 Cells Using Real-Time Quantitative RT-PCR

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ABSTRACT

HL-60 cell line offers an interesting model to study apoptosis. Our aim was to detect the expressions of three apoptotic genes (BCL-2, BAD and BAX) using Real-Time quantitative polymerase chain reaction (PCR). HL-60 cells were incubated with 1,25 $(OH)_2D_3$ (5 x 10^{-8} M). RNA portions were isolated and LightCycler analysis were performed on cDNA samples. 1,25 $(OH)_2D_3$ incubated cell portions were compared to nontreated portions. We observed down-regulated levels of three genes at the end of the 12 hours. Gene expression ratios were 0.98 for BAX, 0.41 for BAD, 0.81 for BCL-2. For the first time, this study represents the expression levels of these three apoptosis related genes together during early phase (12 hours) of 1,25 $(OH)_2D_3$ treatment of HL-60 cells. Data indicate that expression differences of these apoptosis genes take place so early in exposure to 1,25 $(OH)_2D_3$. This optimised strategy would be used in rapid understanding of similar apoptotic deregulations.

Key Words: 1,25 $(OH)_2D_3$, Gene expression, HL-60, Apoptosis.

ÖZET

1,25 $(OH)_2D_3$'le Karşılaşan HL-60 Hücrelerinde Apoptozis ile İlgili Genlerin (BCL-2, BAB, BAX) RT-PCR ile Hızlı Analizi

HL-60 hücre hattı apoptozis çalışmak için ilginç bir model teşkil eder. Amacımız üç apoptotik gene (BCL-2, BAD and BAX) ait anlatımları gerçek zamanlı kantitatif polimeraz zincir reaksiyonu (PCR) kullanarak saptamaktı. HL-60 hücreleri 1,25 $(OH)_2D_3$ (5 x 10^{-8} M) ile inkübe edildi. RNA porsiyonları izole edildi ve cDNA örneklerinde LightCycler analizleri gerçekleştirildi. Oniki saat sonunda üç gene ait anlatım azalması gözledik. Gen anlatım oranları BAX için 0.98, BAD için 0.41, BCL-2 için 0.81 idi. Bu çalışma bu üç genin birlikte anlatım oranlarını 1,25 $(OH)_2D_3$ ile işlenmiş HL-60 hücrelerinde erken fazdaki (12 saat) ilk analizini sunmaktadır. Bilgiler bu apoptozis genlerinin 1,25 $(OH)_2D_3$ maruziyetine bağlı olarak anlatım farklılaşmasının çok erken dönemde yer aldığına işaret etmektedir. Bu optimize edilmiş strateji benzer apoptotik düzen degişikliklerinin hızlı anlaşılmasında kullanılabilir.

Anahtar Kelimeler: 1,25 $(OH)_2D_3$, Gen ekspresyonu, HL-60, Apotozis.

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INTRODUCTION

Characteristic cleavages of the DNA fragments have been regarded as a sign of the apoptosis. There are different methods available to detect the apoptosis. Flow cytometry, polymerase chain reaction (PCR), blotting, FISH, immunohistochemistry are among the most widely used. As for targets they are expanding continuosly. Real-Time quantitative PCR is a recently developed method for the rapid and sensitive detection of gene expression[1]. Our aim was to quantification of the apoptosis related gene expression by Real-Time quantitative PCR at the early phase (12 hours) of 1,25 $(OH)_2D_3$ treatment of HL-60 cells. We have selected three genes for this aim: BCL-2, BAD and BAX. BCL-2 inhibits apoptosis by forming dimers with BAX, encoding a proapoptotic protein[2]. Independently, BCL-2 inhibits apoptosis while BAX induces it. Another apoptosis related protein called as BAD meaning that "BCL-2 antagonist of cell death"[3].

MATERIALS and METHODS

We have used vitamin D incubated HL-6O leukemic cell cultures for the induction of these apoptosis related genes. HL-6O cells are sensitive to the induction of apoptosis, despite their relatively high level of BCL-2 expression. 1,25 $(OH)_2D_3$ protects HL-60 cells against apoptosis but down-regulates the expression of BCL-2 gene. This cell line offers an interesting model to study apoptosis[4-6].

In our experiments, 1,25 $(OH)_2D_3$ (5×10^{-8} M) treatment was performed on HL-60 cells for 12 hours in Iscove's modified Dulbecco's medium (IMDM; Sigma Diagnostics, St. Louis, MO, USA) with 10% fetal calf serum (FCS; Bioclear, Wilts, UK). RNA samples were isolated with Qiagen reagents and treated with DNase I. Quantitative Real-time PCR (LightCycler, Roche Diagnostics GmbH, Germany) was performed as we described previously[7,8]. Standard curves were obtained by using serial dilutions of beta-globulin gene (DNA control kit, Roche, Mannheim, Germany). Gene-specific primers were designed and synthesized by TIB MOLBIOL (Berlin, Germany). Primer sequences of selected genes were shown in Table 1. Obtained gene expression values were normalised using a housekeeping gene in both patient and healthy control groups. For this aim HPRT gene levels were used and expression ratios were calculated using the following formula, ratio: observed gene expressions in 1,25 $(OH)_2D_3$ 12 hours treated HL-60 cells/observed gene expressions in nontreated HL-60 cells.

RESULTS

We observed down-regulated levels of BCL-2, BAD and BAX at the end of the 12 hours (Table 2). Down regulated levels of these three genes paralleled the data we established in our previous studies with ATRA incubations of leukemia patients[9].

Table 1. Primer sequences of selected genes

HPRT (hypoxantine phosphoribosyltransferase 1) "housekeeping" gen (GenBank) NM 00194.1	GGCAGTATAATCCAAAGATGGTCA GTCTGGCTTATATCCAACACTTCGT
BCL-2	AGGAAGTGAACATTTCGGTGAC GCTCAGTTCCAGGACCAGG
BAX (BCL-2-associated X protein)	TGCTTCAGGGTTTCATCCAG GGCGGCAATCATCCTCTG
BAD (BCL-2 antagonist of cell death)	GAGTGAGCAGGAAGACTCCAGC TCCACAAACTCGTCACTCATCC

Table 2. Validation of relative gene expression by quantitative fluorescent PCR

Genes	Chromosome location	Accession number (GenBank)	Ratio
BAX (BCL-2-associated X protein)	19q13.3-q13.4	L22474	0.98
BAD (BCL-2 antagonist of cell death)	11q13.1	AK023420	0.41
BCL-2	18q21.3	M14745	0.81

* Ratio: observed gene expressions in 1,25 $(OH)_2D_3$ treated HL-60 cells for 12 hours/observed gene expressions in nontreated HL-60-cells.

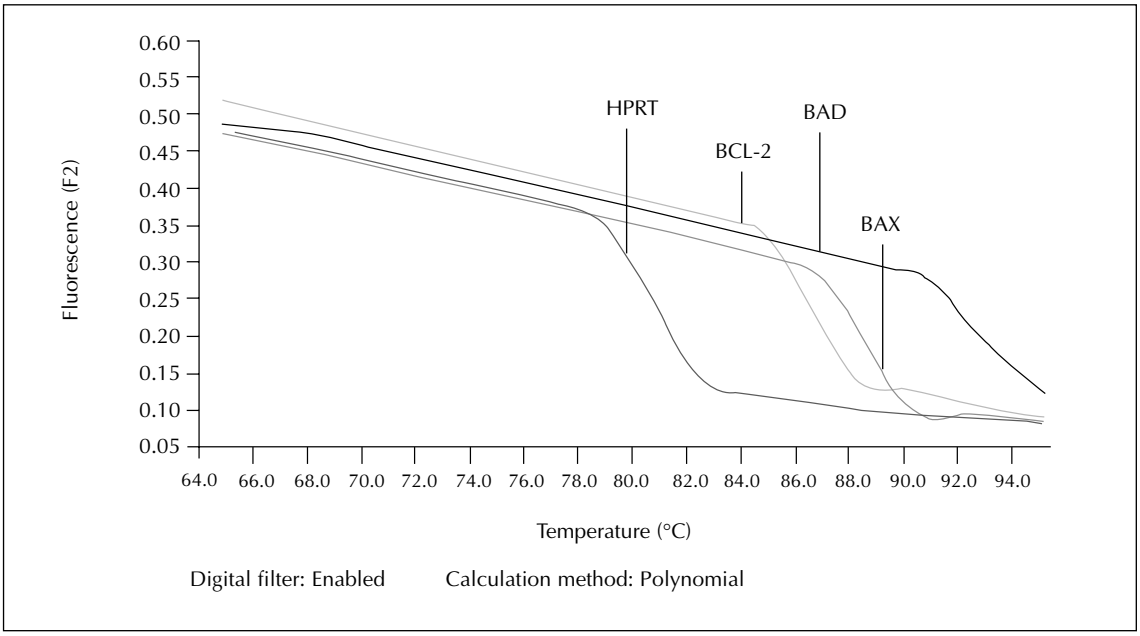

Figure 1. Spesific amplifications of the studied genes. Melting curve analysis of the amplification reactions demonstrating the gradual change in fluoresence as temperature increases. The rapid falls indicates the spesific products that melts at spesific temperatures as 86°C for BCL-2, 92°C for BAD, 89°C for BAX, 81°C for HPRT.

DISCUSSION

This study represents for the first time the expression levels of these three apoptosis related genes together (BCL-2, BAX, BAD) during early phase (12 hours) of 1,25 $(OH)_2D_3$ treatment of HL-60 cells. Data indicate that expression differences of these apoptosis genes takes so early in exposure to 1,25 $(OH)_2D_3$. This study model can be used in rapid understanding of similar apoptotic deregulations.

REFERENCES

1. Ferre F, Marchese A, Pezzoli P, Griffith S, Buxton E, Boyer V. Quantitative PCR: an overview. In: Mullis KB, Ferre F, Gibbs RA (eds). The Polymerase Chain Reaction. Boston: Birkhauser, 1994;67-88.
2. Mitchell KO, Ricci MS, Miyashita T, et al. Bax is a transcriptional target and mediator of MYC-induced apoptosis. Cancer Res 2000;60:6318-25.
3. Yang E, Zha J, Jockel J, Boise LH, Thompson CB, Korsmeyer SJ. Bad, a heterodimeric partner for Bcl-X(L) and Bcl-2, displaces Bax and promotes cell death. Cell 1995;80:285-91.
4. Chatterjee D, Han Z, Mendoza J, Goodglick L, Hendrickson EA, Pantazis P, Wyche JH. Monocytic

differentiation of HL-60 promyelocytic leukemia cells correlates with the induction of Bcl-xL. Cell Growth Differ 1997;8:1083-9.

5. Tudor G, Aguilera A, Halverson DO, Laing ND, Sausville EA. Susceptibility to drug-induced apoptosis correlates with differential modulation of Bad, Bcl-2 and Bcl-xL protein levels. Cell Death Differ 2000;7: 574-86.
6. Cotter TG, Fernandes RS, Verhaegen S, McCarthy JV. Cell death in the myeloid lineage. Immunol Rev 1994;142:93-112.
7. Savli H, Aalto Y, Nagy B, Knuutila S, Pakkala S. Gene expression analysis of 1,25$(OH)_2D_3$ dependent differentiation of HL-60 cells-a cDNA array study. Br J Haematol 2002;118:1065-70.
8. Savli H, Karadenizli A, Kolayli F, Gundes S, Ozbek U, Vahaboglu H. Expression stability of six housekeeping genes: a proposal for resistance gene quantification studies of *Pseudomonas aeruginosa* by real-time quantitative RT-PCR. J Med Microbiol 2003;52:403-8.
9. Savli H, Sirma S, Nagy B, Aktan M, Dincol G, Salcioglu Z, Ozbek U. Verification of the apoptosis related genes during all-trans-retinoic acid treatment in acute promyelocytic leukaemia patients. Exp Mol Med 2003;35:454-9.

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