Application of FLUPD-DD-PCR to the study of mRNA expression of glioma cells cultured under the condition of serum starvation

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Abstract Fluorescently labeled universal primer directed differential display polymerase chain
reaction technique (FLUPD-DD-PCR), an improved DD-PCR, is reported for the study of mRNA expression of glioma cells cultured with the serum starvation. The fluorescently labeled universal primer (FLUP) facilitates analysis of PCR products on an auto-sequencer. Compared with the traditional DD-PCR, FLUPD-DD-PCR has advantages of separation of target bands and measurement of mRNA expression quantitatively by adopting Cy5-labeled universal primer. And then the silver staining method has been used to recover the bands for use as template in re-PCR. In this study, 4 differential ESTs, of which 3 are novel ESTs, have been obtained. This work indicates some special novel genes, which can be induced to express under serum absent condition, are involved in coping with serum starvation stress on glioma cells growth.

Keywords: FLUPD-DD-PCR, silver staining, serum starvation.

Differential display polymerase chain reaction technique (DD-PCR) was developed by Liang and Pardee in 1992[1]. This technology has been traditionally utilized by isotope labeled primers with some disadvantages, such as time consuming, difficulties in identification of the target bands and measurement of the quantitative difference between the studied panels. Due to these problems, we tried to improve it using both a florescently labeled universal primer method and silver staining method in identification and re-amplification of bands (the principle of FLUPD-DD-PCR is shown in fig. 1)[2, 3]. In this study, a differential display of expression of the glioma cells cultured under normal condition and serum starvation condition was compared using FLUPD-DD-PCR and silver staining method respectively. As serum was assumed to be an important factor in the tumor growth and apoptosis process, we detected the genes involved in this event and found that 4 ESTs were specially expressed under the condition of serum starvation treatment. Our work also demonstrates that FLUPD-DD-PCR can be used to analyze gene expressions efficiently and accurately.

1 Materials and methods

(i) Primers. Fluorescently labeled universal primer (FLUP), 5'-cy5-CTCACGGATCCGTCGA-TTTT-3', was produced by Pharmacia Company Ltd. Reverse transcription primers (RTP) are as follows:

5'-CTCACGGATCCGTCGATTTTATTTTTTTTTTTA-3',
5'-CTCACGGATCCGTCGATTTTATTTTTTTTTTTG-3'.

The underlined reverse transcription primers have the same sequence as the universal primer. Random primer (RP) is 5'-TACCTAAGCG-3'. Both the RTPs and RP are synthesized by GibcoBRL Company Ltd.

(ii) Cell culture and treatment. The A172 cells, divided into two groups, were cultured in 3.5 cm diameter plates with F12 medium, 10% fetal serum, 5% CO₂ at 37°C until the cell density reached 70%. One group was treated with F12 medium without serum and the other one served as control with F12 containing 10% fetal serum for 20 h respectively.

(iii) cDNA preparation. Total RNA was exacted with RNA Trizol (GibcoBRL) followed by the treatment with DNaseI to get rid of DNA. 1 µg of extracted total RNA was used as template for reverse transcription and 50 pmol/L UP-T8GA as primer in this assay. The reaction was performed with 20 µmol/L dNTP, 300 units MMLV at 42°C for 1 h, and then at 95°C for 5 min.

(iv) FLUPD-DD-PCR. One microliter of reversibly transcripted product was used as FLUPD-