Crystal structure determination of proteins involved in ALK mediated lymphoma

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ABSTRACT

Cancer is abnormal and uncontrolled cell division and can be benign or malignant. It is the principle cause of worldwide mortality. G0/G1 switch 2 (G0S2) is a small and basic protein involved in pushing cells into the G1 phase of the cell cycle. Therefore, the increased expression of this gene is correlated with increased cells proliferation and its prolonged life span. The up-regulation of the expression of G0S2 is hypothesized to be relevant to the development of cancer. However, the structure of G0S2 has yet been published. In this study we attempt to use the cloning of G0S2 to overexpress this protein and determine its structure by X-ray crystallography. Subsequently, it will help in the development of therapeutic measures against cancer.

INTRODUCTION

G0S2 is a small and basic protein (11.3 kDa) involved in the efficiency of the G0 – G1 cell cycle transition. However, the precise regulatory function of the G0S2 protein in cell growth and differentiation still remains unclear and the structure would be beneficial in the study for cancer therapeutics. The present study aims to clone the G0S2 gene from cancerous lymphocytes and express this protein in a prokaryotic expression system.

MATERIALS AND METHODS

G0S2 gene from SUDHL1 and SUPM2 lymphocytes cell lines was amplified by Polymerase Chain Reaction (PCR). The forward primer, 5’-CGG GGA ATT CAT GGA AAC GGT CCA G-3’ with an EcoRI site (underlined) and the reverse primer, 5’-CAA CTC GAG CTA GGA GGC GTG C–3’ with an XhoI site (underlined) were designed. The conditions for the PCR were hot start at 80°C for 10 minutes, 30 cycles at 94°C for 10s, 64°C for 10s and 72°C for 60s using both the forward and reverse primers. The G0S2 gene fragment was cloned into pET32a-M vector and transformed into Escherichia coli BL21 (DE3) competent cells for protein expression.

RESULTS

The PCR colonies were verified using 0.7% agarose gel, Fig. 1.

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The protein fractions of overexpressed G0S2 were verified using 12% SDS-PAGE gel, Fig. 2.

**Figure 1.** 0.7% Agarose gel showing the positive clone obtained from colony PCR. Lanes 1 to 10 – Clones 1 to 10, M – 100 bp DNA marker

**Figure 2.** Repeated prokaryotic expression analysis of the recombinant protein on 15% SDS-PAGE with Coomassie blue staining. M – Marker, Lane 1 - whole cell fraction (0 mM IPTG), Lane 2 - whole cell fraction (1 mM IPTG), Lane 3 - whole cell fraction (0.5 mM IPTG), Lane 4 - whole cell fraction (0.3 mM IPTG), Lane 5 - soluble fraction (0 mM IPTG), Lane 6 - soluble fraction (1 mM IPTG), Lane 7 - soluble fraction (0.5 mM IPTG), Lane 8 - soluble fraction (0.3 mM IPTG)
Affinity chromatography protein fractions were verified using 15% SDS PAGE gel, Fig. 3.

**Figure 3.** SDS-PAGE showing the affinity chromatographic purification of g0s2 protein. M – Protein marker, Lane 1 – crude sample, Lane 2 – flow through, Lane 3 – wash 1 with 1 M NaCl, Lane 4 – wash 2 with 1 M NaCl, Lane 5 – wash 3 with 200 mM NaCl, Lane 6 – wash 4 with 200 mM NaCl, Lane 7 – Eluted solution 1, Lane 8 – Beads, Lane 9 – Eluted solution 2

**DISCUSSION**

**Molecular Cloning of G0S2**

SUDHL1 lymphocytes cell line was chosen as it had higher cDNA concentration and purity after PCR amplification. Double digestion of vector pET32a-M and G0S2 using EcoRI and XhoI endonuclease, and ligation were successful due to high bacterial count on selection plates. Dideoxy sequencing confirmed the presence of G0S2 in the recombinant plasmid. However, two single base substitutions were observed after sequence alignment. This translated to a single amino acid substitution in G0S2 protein sequence.

**Small Scale Protein Expression**

Being a small protein, it is a challenge to produce a high yield G0S2 protein to be visualized. High IPTG of 0.5 mM was needed to induce G0S2 expression. 15% SDS PAGE gel produced a better resolution of proteins migration compared to 12% SDS PAGE gel. Presence of G0S2 validated due to the confirmation of presence of His-tag using affinity chromatography. However, mass spectrometry was not able to confirm the presence of G0S2. This is due to an intense background which indicates impurity and concentration of protein below the threshold value.
REFERENCES


