Recombinant production of Annexin V protein for apoptosis detection to monitor cancer therapy

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Abstract

Introduction:

Apoptosis or programmed cell death (PCD) plays an important role not only in physiology but also in pathology. One of the most prominent features of apoptosis is externalization of phosphatidylserine (PS), which in healthy cells are located in the inner leaflet of the plasma membrane. PS-externalization have made a well-explored phenomenon to image cell death for diagnostic and therapeutic purposes. Since many drugs induce a therapeutic effect through the activation of PCD in target cells, imaging of cell death offers a direct way to image therapy response. Moreover, failure of therapy is frequently a result of resistance against apoptosis. Therefore detection and quantification of apoptosis are some of the significant clinical value for diagnosis and assessment for therapeutic efficacy. In this research we emphasize the expression and purification of recombinant polyhistidine-tagged human annexin V protein that binds to PS with high affinity and has been developed as a molecular imaging agent to measure cell death in vitro and in vivo in animal models and in patients with cardiovascular diseases and cancer. We also describe conjugation of this protein to the Fluorescein isothiocyanate (FITC) fluorophore for the detection of apoptotic cells by flow cytometry or fluorescence microscopy.

Methods and Results:

In this project we transformed recombinant plasmid including annexin V gene into competent BL21 strain. after an overnight incubation at 37°C in LB medium,1 mL of that was used to inoculate 100 mL of TB culture.

The expression of annexin V was induced with 1 mM IPTG (isopropyl-b-D-thiogalactopyranoside).

The expression level of annexin V was evaluated by SDS-PAGE. Recombinant annexin V was expressed and purified to high yields. FITC as the fluorescent conjugate and a signal detector was used to attach to annexin V. we detected apoptotic cells in culture by annexin V-FITC produced probe in real time, using fluorescence microscopy and flow cytometry.

Conclusions:

Annexin V staining is a simple and widely used method for detection of apoptosis in a rapid and highly quantitative manner for both early monitoring of therapy capability and assessment of disease development.

Key words: Apoptosis, Annexin V, PS, FITC, Cancer therapy

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