Cloning, Expression and Purification of Human Folate Receptor Alpha Precursor for Bioanalytical Applications for Cancer Research

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Introduction: Recently, Lima et al. (2010) reported the possibility of the use of biosensors for biomarkers detection in urine, blood or other bodily fluids, facilitating the diagnosis of cancers in terms of operation, cost, and speed. Cancer cells have specific receptors with high affinity to folate and are highly replicative; these cells are dependent on the supply of reduced folate. With rare exceptions, alpha folate receptor (FRα) is induced selectively in cancerous tissues and rarely expressed in normal cells, thus being highly sensitive and specific. FRαs are extracellular proteins with specific biochemical properties, which can be exploited by marking strategy for detection and treatment of the disease. This project proposes cloning, expression and purification of the recombinant FRα for subsequent immobilization on a microsystem, mimicking a cancer cell, becoming an analytical standard for studies with biosensors recently developed. Material and Methods: Human FRα fragment was obtained by polymerase chain reaction and cloned into a plasmid vector pET28a via NdeI and XhoI sites. The expression plasmid was transformed into E. coli BL21 (DE3) cells for rFRα expression, and induced with IPTG. The recombinant product had a His-tag on the N-terminus. Expression, solubility, and purity of rFRα were examined with 15% SDS-PAGE. Results and Discussion: The recombinant protein was found to be mostly in the pellet after cell lysis. Refolding protocols were performed, which partially helped on the solubilization. For a primary purification a nickel-chelating column was used. Conclusions: The expression system of rFRα will be optimized since soluble protein was obtained in low quantity and after a resolubilization protocol. Yet, bioanalytical characterization of rFRα is useful for understanding the role of this protein in the development of tumor tissues and development of drugs that potentially inactivate this protein, such as the anti-folates.

Key words: Cloning, Heterologous Expression, Folate Receptors, Tumor Biomarker.