**Design of a single step chromatographic strategy for the isolation of the cancer-associated antigen STEAP1**

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**Highlights**

* The extraction of STEAP1 was optimized to increase the concentration of total protein.
* A reproducible strategy for STEAP1 isolation and buffers composition were established.
* STEAP1 was isolated by HIC in a single step and co-eluted with residual of contaminants.

**1. Introduction**

The Six-Transmembrane Epithelial Antigen of the Prostate 1 (STEAP1) is a cell-surface antigen overexpressed in several types of cancer, namely in prostate cancer [1]. In normal cells the levels of STEAP1 are low or absent [1, 2]. Considering its secondary structure and location in the cell membrane, it has been suggested a role in intercellular communication between tumor cells [2, 3]. These features highlight STEAP1 as a promising therapeutic target. The main goal of this work is to express high levels of STEAP1 protein from LNCaP cells and isolate the target protein in a native form by traditional hydrophobic interaction matrices.

**2. Methods**

LNCaP cells were maintained in a humidified chamber with controlled atmosphere until 90-100% confluence. These cells were lysed onto RIPA buffer or an in-house buffer for nuclear and cytosolic extraction of proteins. This procedure was fully optimized regarding the volume of buffers and the concentration of proteins to further enhance the yield of the isolation process and immunoblot experiments. Total protein samples were loaded onto a butyl sepharose column and the manipulation of ionic strength was used to promote a complete retention of proteins to the matrix and a selective fractionation of impurities. The purification step was monitored by SDS-PAGE and STEAP1 was detected in dot-blot analysis or a single band in western blot with approximately 39 KDa [1]. Mass spectrometry was also used to confirm the identity of the target protein. Lastly, circular dichroism was used to infer if the concentration of ammonium sulphate effectively influences the arrangement of secondary structure of STEAP1 or promote interactions between the target protein and unspecific compounds.

**3. Results and discussion**

Once STEAP1 is overexpressed in LNCaP, we extracted total protein and also both cytosolic and nuclear fractions. The western blot analysis showed an increased expression of STEAP1 in cytosolic fraction, with similar levels to total protein extracts. These aliquots were applied in the chromatographic studies. The main results demonstrated that STEAP1 was fully captured in butyl sepharose matrix since the concentration of ammonium sulphate in the binding buffer was 1500 mM and 2000 mM. However, the elution profile denoted low selectivity, once STEAP1 was detected at similar levels in distinct steps of the elution salt gradient, namely 10 mM Tris-HCl and H2O. Likewise, these samples presented a considerable background of impurities that co-elute with the target protein. Interestingly, STEAP1 was not retained at 1000 mM and 1250 mM of salt. This result could be explained considering the presence of a non-ionic detergent in the lysis buffer which condition the exposure of the hydrophobic domains of the predictive six-transmembrane structure of STEAP1. This phenomenon clearly affected the interaction of the target protein with the chromatographic matrix and explains the reason behind the significant retention of several impurities at lower concentrations of ammonium sulphate. Otherwise, as the salt concentration in the binding buffer increases, the interactions between STEAP1 and butyl sepharose are consequently promoted. Nevertheless, these findings were valuable because it was conceivable to isolate STEAP1 in a single chromatographic step with residual amount of interfering biomolecules.

**4. Conclusions**

This work established for the first time a reproducible chromatographic procedure for the isolation of human STEAP1 with high expression levels and significant grade of purity. These optimized conditions will allow that samples containing the target protein may be further used for a detailed biochemical and structural characterization of STEAP1 and later development of molecules that were able to block its oncogenic functions.

**References**

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