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The presence of bone metastases in patients with castration-resistant prostate cancer (CRPC) has a major impact in decreasing overall survival

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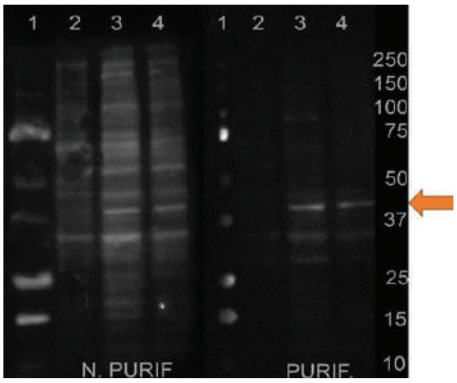
The presence of bone metastases in patients with castration-resistant prostate cancer (CRPC) has a major impact in decreasing overall survival1. Electrochemical biosensors are analytical tools that exhibit several benefits to the diagnostic area because they offer low cost, portability, high sensitivity and precision2. Regarding immunosensors, in which the recognition element is an antibody, the specificity is increased because it is a molecule capable of recognizing only a single antigenic determinant3. In this study, the multiple tolerization subtractive immunization (MTSI) was used to obtain murine monoclonal antibodies (mAbs) aiming at recognize new biomarkers present in metastatic cells derived from PC-3 cell line. MTSI is based on increasing of the cellular and humoral immune response against relevant, rare or weakly immunogenic antigens present in pathological conditions such as cancer and infections diseases4,5. The cyclophosphamide acts as immunosuppressive drug, eradicating T and B lymphocytes reactive to immunodominant antigens of no interest6,7. After cell fusion technique, cloning and screening of the hybridomas8, a mAb was identified capable of recognizing a 45 kDa protein in both extracts of the prostatic metastatic cell lines (PC-3 and LNCaP). This antigen is possibly located in the nuclear portion of malignant cells as verified by an indirect immunofluorescence assay. In addition, this biomolecule demonstrated complement-dependent cytotoxicity activity against PC-3 cells of manner dose-response fashion using propidium iodide as dead cells marker. The next step consists of immobilizing this mAb on a platform based on layer-by-layer technique and evaluating the sensitivity and specificity of the immunosensor in detecting circulating prostatic tumor cells.



Chemiluminescent western blotting assay to identify the approximate protein weight recognized by the antibody derived from hybridoma clone mAb50. 1: prestained protein ladder, 10 to 250 kDa. 2: Non-tumorigenic cell line RWPE- 3: tumorigenic cell line LNCaP. 4: tumorigenic cell line PC-3. Left side shows the mAb non purified and right side shows the mAb purified. The arrow indicates the identification of a protein of approximately 45 kDa.

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