Determination of DAMP Signals from Immunogenic Cell Death Biomarkers for Anticancer Drug Development Studies with Nano Electrochemical Aptasensor

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Abstract

Numerous studies are conducted to develop new cancer treatments worldwide. Triggering immunogenic cell death (ICD), providing immune response against cancer antigens by creating danger signals called "damage-associated molecular patterns (DAMPs)," is also a remarkable treatment approach. Although many studies underline that ICD triggering chemotherapeutics are highly potential drug candidates, a limited number of ICD triggers have been identified. It is extremely important to be able to determine the formation of DAMP signals effectively and quickly in order to screen the activities of candidate molecules [1]. It is essential to develop methods that are fast, sensitive, cost-effective and provide quantitative results compared to existing methods (microscopic, immunoblot, ELISA) used in determination of DAMP signals. Electrochemical biosensors form the basis of compact and commercializable chip designs with features such as being cheap and portable. Aptamers application, which are defined as artificial antibodies, in biosensor studies gained momentum due to the disadvantages of using antibodies as sensing parts in biosensors: high cost, difficulty in production and low stability. In this study, sensitive, reliable, low-cost, nanomaterial-enriched aptasensor systems are developed for DAMP signals detection at cell level. HMGB1 [2] and CALR [3] are determined as model markers of ICD. In the first stage, label-free electrochemical aptasensors were designed for protein detection. Selectivity of the aptasensors are monitored with HSP70 and VEGF. To increase biological molecule binding capacity of sensor surfaces, electrodes are enriched with nanofibers using electro-spinning method. At each stage, electrochemical changes between electrode/electrolyte will be monitored by Electrochemical Impedance Spectroscopy (EIS). In the second step, Oxaliplatin is used in three different cell lines to generate DAMP signals. Prototype aptasensors are applied at cell and supernatant level, for calreticulin (ecto-CALR) expressed on cell surface detection and released HMGB1. Cisplatin-treated and untreated negative control cells and supernatants are used to control selectivity. By taking advantage of the specificity and selectivity brought by the aptamers, and the high binding capacity provided by nanofibers due to its large surface area, a sensor system specific to DAMP signals allowing label-free analysis that does not require preprocessing and is easy to apply, was developed in this study as an alternative method to the antibody-based methods.

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