Breast cancer: Biomarkers and Biosensors

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Introduction

Breast cancer remains one of the leading causes of cancer-related deaths, accounting for approximately 521,000 deaths per year. Although technology and research have improved rapidly in recent years, breast cancer diagnosis remains a challenge. This is not only because of late diagnosis, especially in developing countries where sophisticated techniques and equipment needed for proper diagnosis are still lacking, but also because of the time-consuming steps involved in all the tests that are performed to get a diagnosis. Therefore, there is an urgent need for early stage diagnosis solutions. This means finding specific biomarkers and developing better ways to screen for them cheaply and quickly.

Overall, this project proposes the development of a biosensor that accounts for aptamers and/or proteins that can specifically detect the different subtypes of breast cancer, using phage display and SELEX approaches in a cellulose immobilization carrier that gives a colorimetric response. Once this is achieved, this device could be used as a POC device in large-scale population screening and surveillance programs.

Goals

I. Selection and design of the biomarker elements: aptamers and peptides against breast cancer;

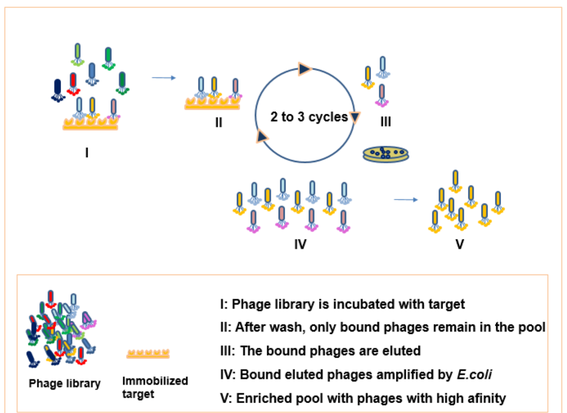
II. Selection and chemical modification of the immobilization support;

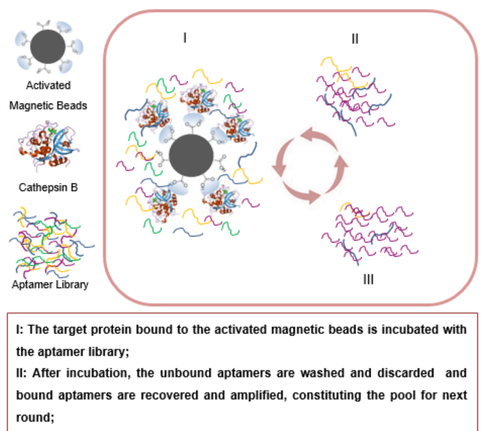
III. Development and construction of the biosensor;

IV. Color development and signal optimization within clinical range.

Methodology

I. Use of the Phage Display technique to select peptides against a breast cancer cell line (left) and the SELEX technique to select aptamers against a target protein (right).



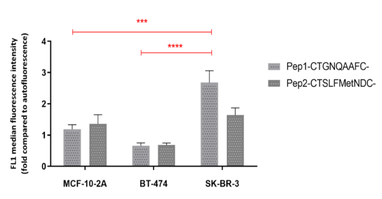


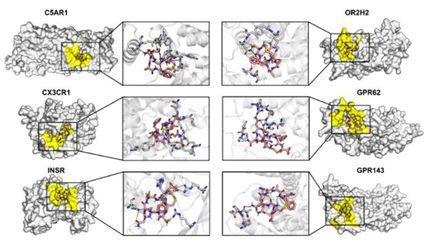
II. The biosensor assembly started by washing the cellulose substrate, followed by a chemical modification with a silane and, finally, the aptamer immobilization by thiol bond.

Using a common protein dye, a color response in the presence of a protein target, associated with breast cancer malignancy, allows its semi-quantification by naked eye and quantification by RGB/HSB coordinates system with Image J software.

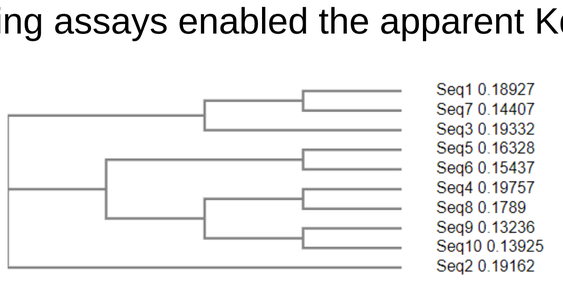
Results

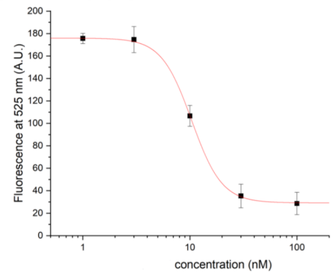
A new peptide was isolated against the breast cancer cell line, Sk-Br-3. Cytometry assays showed good selectivity for the target cells and, using bioinformatic tools, six potential membrane receptors have been identified.



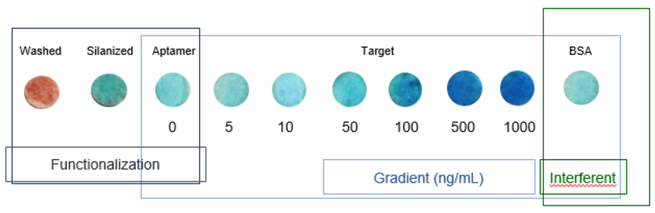


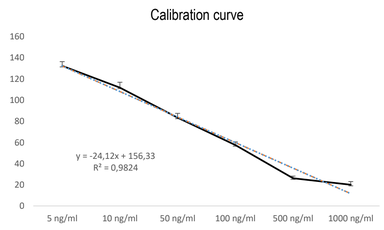
Using SELEX against the target protein Cathepsin B, an aptamer with potential for breast cancer screening was isolated. NGS and bioinformatics was used to identify the best candidate (seq 8) and binding assays enabled the apparent Kd determination





After biosensor assemble, the protein dye allowed to distinguish the different steps of the biosensor assembly (washed paper vs silanized paper vs aptamerized paper), as well as increasing concentrations of the target, within the clinical range, in a linear response for the Red coordinate in the Image J software.





Currently, other approaches are being explored for these new biomarkers.

Acknowledgments

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