PROTEOMICS APPLICATION IN BREAST CANCER

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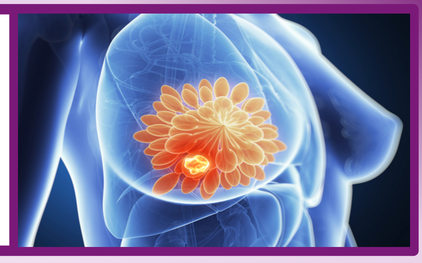


INTRODUCTION

Biomarkers are detectable molecules that change depending on the physiological state of the organism, they are usually proteins. To find a good biomarker first we need to find it and then validate it. Proteomic techniques are useful in order to get an ideal biomarker.

OBJECTIVES

- ✓ Knowing the steps to be followed to find a specific biomarker for a certain disease.
- ✓ Understanding the most used proteomic techniques.
- ✓ Interpreting papers that use these techniques to discover potential biomarkers that detect breast cancer.

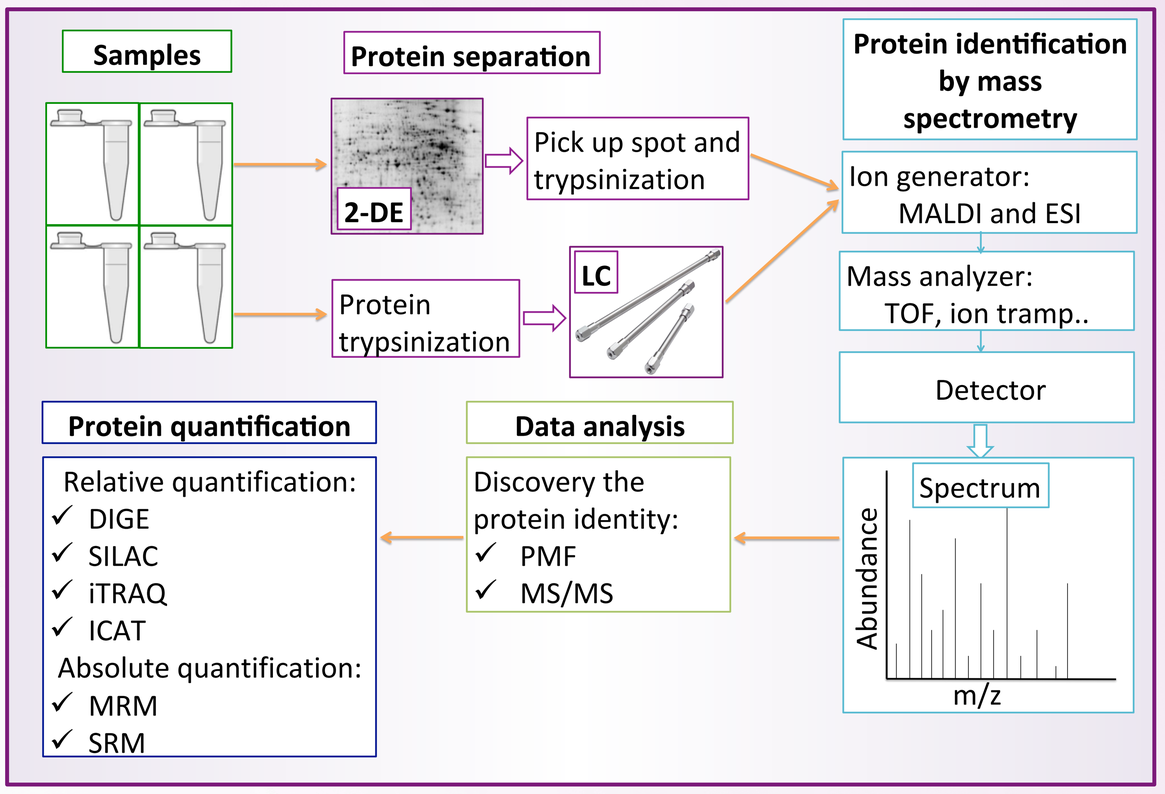
Stages for the biomarker research

Stages	Samples	Process of samples	Number of targets	Number of samples
Discovery Identify target biomarkers	Cells, tissues and biological fluids	Depletion and high sample fractionation	1000	10
Qualification Differential expresión confirmation	Biological fluids and little biological variability	Depletion and mild sample fractionation	30 & 100	10
Verification Targets specifity valoration	Biological fluids and high biological variability	Depletion and mild sample fractionation	10	100
Validation Sensibility assessing and method optimization	Biological fluids and high biological variability	No treatment	4 & 1	1000

IDEAL BIOMARKER

[9]

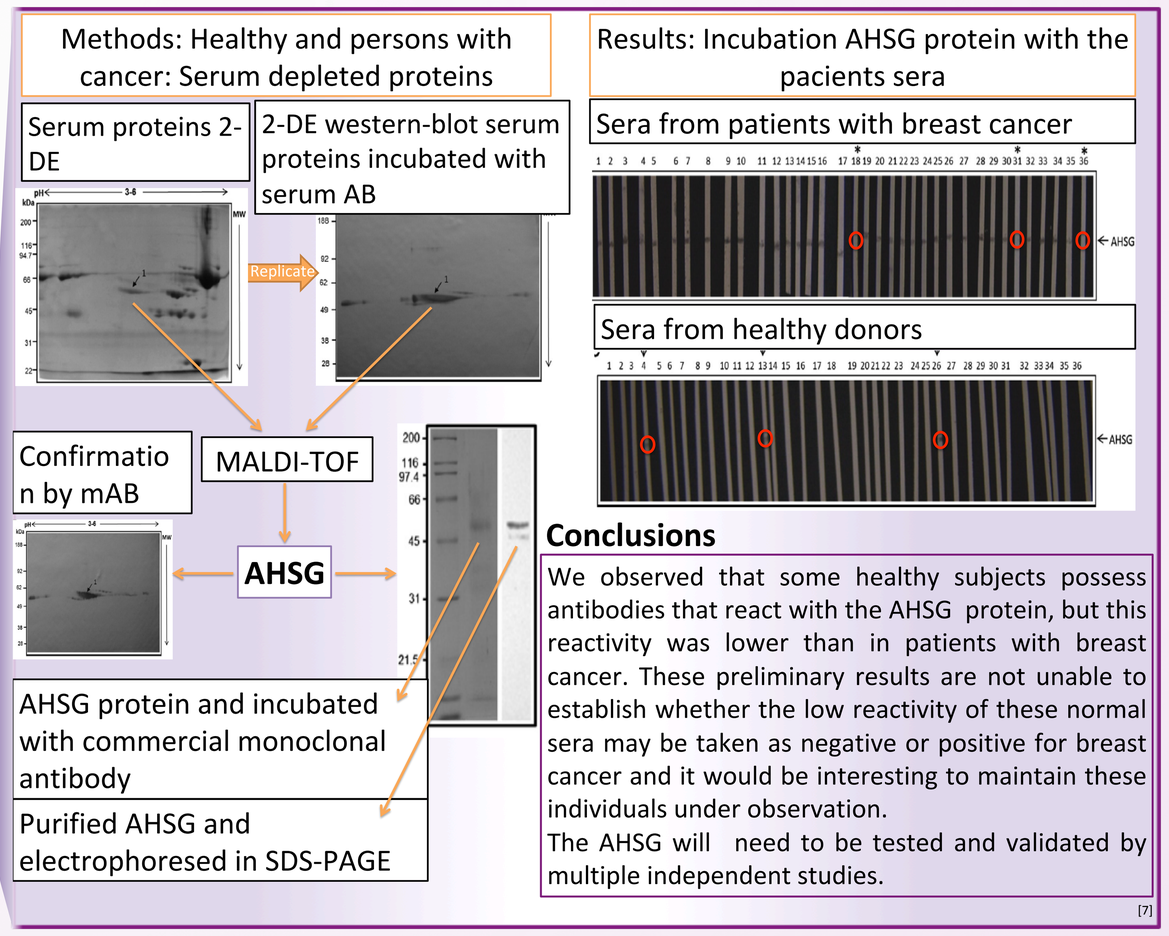
Proteomics techniques used in the biomarker research



Eight proteomic studies to identify potential breast cancer biomarker

Case	Cancer type	Sample type	Proteomic techniques	Potencial biomarkers	Year
1 [1]	Invasive carcinoma of no special type (NST)	Tissues: Fibroadenoma vs NST	2-DE→ MALDI-TOF	Calreticulin, HSP-70, triosophosphate isomerase I, pyruvate kinase M1 and β-tubulin chain 1 o 5	2004
2 [2]	Invasive carcinoma of no special type (NST)	Tissues: Healthy vs NST	2-DE→ MALDI TOF/TOF	α-1-antitrypsin, catepsin D, translationally controled tumor-associated protein	2006
3 [3]	Invasive carcinoma of no special type (NST)	Serum: Healthy vs NST	SERPA: 2-DE+western-blot (cultivate in a MCF-7) and MALDI-TOF	26 immunoreactives proteins: HSP60, prohibitin (tumor supprsesor), β-tubulin, haptoglobin and peroxiredoxina-2	2008
4 [4]	Differenst stages of invasive carcinoma of no special type (NST)	Serum: Healthy vs different stages of the tumor pacients	Albumin depletion and after 2-DE→ MALDI-TOF	α-1-antitrypsin and haptoglobin	2009
5 [5]	Stage I of invasive carcinoma of no special type (NST)	Serum: Healthy vs NST type I	iTRAQ and LC-TOF	Find 23 desregulated proteins: Actin protein, SPARC-like protein 1, haptoglobin…	2011
6 [6]	Stage IV of breast cancer	Serum: Healthy vs breast cancer pacients	Transferrin purification by affinity column and HPLC MS/MS	Fibrinogen chains, fibronectin-1, complement components	2014
7 [7]	Early stage breast cancer	Serum: Healthy vs breast cancer pacients	SERPA: 2-DE + western blot (cultivate in pacients proteins) and MALDI-TOF	α-HS-glicoproteína (AHSG)	2014
8 [8]	Monitoring a breast cancer cell line treated with retinoid acids		2-DE→ MALDI-TOF	Some of the proteins that were induced to be treated with retinoid acids: HSP 27, cytokeratin…	2015

Case 7: identification of a potencial biomarker for the early stage breast cancer



Conclusions

We observed that some healthy subjects possess antibodies that react with the AHSG protein, but this reactivity was lower than in patients with breast cancer. These preliminary results are not unable to establish whether the low reactivity of these normal sera may be taken as negative or positive for breast cancer and it would be interesting to maintain these individuals under observation.

The AHSG will need to be tested and validated by multiple independent studies.

[7]

CONCLUSIONS

- ✓ The proteomic techniques are based on, first the separation of proteins from the sample, given by ”gel-based" (2-DE) or "gel free" (LC) techniques. Second its identification yielded by the mass spectrometer and its informatics analysis. Finally, we can do a quantitative protein comparison chosen between two different samples.
- ✓ In order to find an ideal biomarker for a disease, it should be discovered, qualified, verified and validated.
- ✓ Eight of the studies chosen, the oldest ones use less sensible techniques and always look at the infiltrating ductal breast cancer; as the studies are more current the techniques used are more precise and are able to detect low abundance proteins and use more variety of breast cancer subtypes. On the other hand, we can see that different studies conclude with the same potential biomarkers, which is of interest to have them as a reference.
- ✓ Immunoproteomic is a strong tool to detect novel tumour antigens, which cause a humoral immune response in patients with breast cancer. These antigens and/or its circulating antibodies may be very clinically usefull and a possible potential diagnostic biomarker. In this case we have a potential biomarker to detect early stage breast cancer.

DISCUSSION

- ✓ The detection of minor proteins would be of great interest in the search of new biomarkers. Enhancing the separation techniques of proteins would enable the detection of polyvalent proteins in the tissues and would give rise to an improvement in the sensibility detection of such proteins.
- ✓ Although common biomarkers have been detected in different investigations, most of them are inflammatory proteins or are in abundance in the blood, so they would be altered in most diseases. In order to be able to conclude that these biomarkers are completely specific for breast cancer we would need thorough studies comparing this biomarker with other disease subtypes. This comparison could establish the specificity of the biomarker.
- ✓ It should be considered to elaborate a biomarker profile that can differentiate between the different types of cancer and its different stages. Biomarker profile would allow having a specific and personalised treatment for each patient depending on the breast cancer subtype.

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