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**INTRODUCTION**

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**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**

<table>
<thead>
<tr>
<th>Stages</th>
<th>Samples</th>
<th>Process of samples</th>
<th>Number of targets</th>
<th>Number of samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Discovery</td>
<td>Cells, tissues and biological fluids</td>
<td>Depletion and high sample fraction</td>
<td>1000</td>
<td>10</td>
</tr>
<tr>
<td>Identification</td>
<td>Biological fluids and little biological variability</td>
<td>Depletion and mild sample fraction</td>
<td>30 &amp; 100</td>
<td>10</td>
</tr>
<tr>
<td>Qualification</td>
<td>Biological fluids and little biological variability</td>
<td>Depletion and mild sample fraction</td>
<td>10</td>
<td>100</td>
</tr>
<tr>
<td>Differential expression confirmation</td>
<td>Biological fluids and high biological variability</td>
<td>Depletion and mild sample fraction</td>
<td>4 &amp; 1</td>
<td>100</td>
</tr>
<tr>
<td>Verification</td>
<td>Biological fluids and high biological variability</td>
<td>No treatment</td>
<td>No treatment</td>
<td>1000</td>
</tr>
</tbody>
</table>

**IDEAL BIOMARKER**

**Eight proteomic studies to identify potential breast cancer biomarker**

<table>
<thead>
<tr>
<th>Case</th>
<th>Cancer type</th>
<th>Sample type</th>
<th>Proteomic techniques</th>
<th>Potential biomarkers</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Invasive carcinoma of no special type (NST)</td>
<td>Tissue: Fibroadenoma vs NST</td>
<td>2-DE MALDI-TOF</td>
<td>Calreticulin, HSP-70, trisoposphate isomerase I, galectin 3, β-catenin</td>
<td>2004</td>
</tr>
<tr>
<td>2</td>
<td>Invasive carcinoma of no special type (NST)</td>
<td>Tissue: Healthy vs NST</td>
<td>2-DE MALDI-TOF/TOF</td>
<td>36 Protein biomarkers</td>
<td>2006</td>
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<tr>
<td>3</td>
<td>Invasive carcinoma of no special type (NST)</td>
<td>Serum: Healthy vs NST</td>
<td>2-DE western blot (cultivate in MCF-7) and MALDI-TOF</td>
<td>2-DE: statistical differences</td>
<td>2008</td>
</tr>
<tr>
<td>4</td>
<td>Different stages of invasive carcinoma of no special type (NST)</td>
<td>Serum: Healthy vs different stages of the tumor patients</td>
<td>Albumin depletion and after 2-DE MALDI-TOF</td>
<td>2-DE: statistical differences</td>
<td>2009</td>
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<tr>
<td>5</td>
<td>Stage I of invasive carcinoma of no special type (NST)</td>
<td>Serum: Healthy vs stage I</td>
<td>ITRAQ and LC-TOF</td>
<td>25 Protein biomarkers</td>
<td>2011</td>
</tr>
<tr>
<td>6</td>
<td>Stage IV of breast cancer</td>
<td>Serum: Healthy vs breast cancer patients</td>
<td>Transferin purification by affinity column and HPLC/MS/MS</td>
<td>5 Protein biomarkers</td>
<td>2014</td>
</tr>
<tr>
<td>7</td>
<td>Early stage breast cancer</td>
<td>Serum: Healthy vs breast cancer patients</td>
<td>2-DE western blot (cultivate in patients) and MALDI-TOF</td>
<td>15 Protein biomarkers</td>
<td>2014</td>
</tr>
<tr>
<td>8</td>
<td>Monitoring breast cancer cell line treated with retinoids</td>
<td>2-DE MALDI-TOF</td>
<td>Some of the proteins that were induced to be treated with retinoid acids: HSP 27, cytoplasmic..</td>
<td>2015</td>
<td></td>
</tr>
</tbody>
</table>

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

**Methods:** Healthy and persons with cancer: Serum depleted proteins

**Protein separation:**

- 2-DE western-blot serum proteins incubated with serum AB

**Protein quantification:**

- Relative quantification: DIGE, SILAC, ITRAQ, ICAT
- Absolute quantification: MRM, SRM

**Data analysis:**

- Discovery the protein identity: PMF, MS/MS

**Results:** Incubation AHSG protein with the patients sera

**Case:** Sera from patients with breast cancer

**Confirmatio by m Ab:**

- MALDI-TOF: AHSG

**AHSG protein and incubated with commercial monoclonal antibody**

**Purified AHSG and electrophoresed in SDS-PAGE**

**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.

**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 sensitivity 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.

**REFERENCES**


**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 separation 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 sensitive techniques and always look at the infiltrating ductal breast cancer; as the studies are more mature 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.
- Immunoproteomics 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 useful and a possible potential diagnostic biomarker. In this case we have a potential biomarker to detect early stage breast cancer.