Breast cancer is the most common cancer diagnosed among women in the western world and is the leading cause of female cancer death. Triple-negative (TN) breast cancer (about 15% of all breast cancers) is defined by a lack of expression of estrogen (ER), progesterone (PR), and HER2/neu receptors, and is associated with aggressive phenotype and poor prognosis. In contrast to HER2-positive or hormone receptor positive breast cancers, for which targeted therapies do exist, there is no targeted therapy option for triple-negative breast cancer, which underlines the necessity for further characterization of the molecular basis underlying TN mamma carcinomas.

We compared the protein expression pattern of triple-negative breast carcinomas (HER2-, ER-, PR-) versus those being positive for HER2 and negative for the hormone receptors (HER2+, ER-, PR-) by two-dimensional difference gel
electrophoresis (2D-DIGE) and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOFMS). The DIGE technology allows to compare protein expression levels quantitatively.

By this approach, we identified a number of proteins differentially expressed in triple-negative breast cancers in comparison with HER2-positive/hormone receptor negative tumors. Immunohistochemistry and Western blot results supported the validity of our results. The identified proteins, like for example annexins A1 and A2, lactoferrin, NME1-NME2 protein, fibronectin, and L-plastin, may represent potential targets, and, therefore, may lead to the development of new treatment regimes effective in those tumors that fail to express the known targets such as estrogen and progesterone or HER2 receptors.

Publication:

Taking account of the HMGU mission and vision:
Due to the lack of specific treatment for patients with triple-negative breast cancer, there is a need to develop new therapeutic approaches and to advance our understanding of the pathways that drive proliferation of these tumors. Our research aims towards providing the basis for the development of tailored therapy regimes for the individual breast cancer patient that takes into account the tumor’s biological characteristics.

The internal HMGU co-operation partners with whom the Highlight was compiled, if appropriate:
The project was performed in co-operation with the Institute of Radiation Biology and the TU-München, Institute of Pathology.
Identification of differentially expressed proteins in triple-negative breast carcinomas

Institute of Pathology

Triple-negative (TN) breast cancer is defined by a lack of expression of estrogen, progesterone, and HER2/neu receptors, and accounts for about 15% of all breast cancers. This subtype is associated with an aggressive phenotype, poor prognosis, and unresponsiveness to usual endocrine therapies.

**Figure 1**

- 34 frozen breast tumor tissues (15 triple-negative and 19 HER-2/neu-positive / hormone receptor-negative tumors) were examined
- All were invasive ductal carcinomas (IDC), as assessed by H&E staining (Fig. 1A)
- Only tumors negative for the hormone receptors (Fig. 1C+D), and negative for the HER2 receptor (TN group) or positive for the HER2 receptor (HER2+ group) (Fig. 1B), respectively, were involved in the study

*Schulz et al., J Proteome Res 8: 3430, 2009*
Two-dimensional difference gel electrophoresis (DIGE) for quantitative comparison of protein expression

To correct for interindividual variation we used pooled protein extracts from triple-negative (TN) breast tumors and HER2 positive breast cancer samples (HER2+). DIGE analysis was performed for assessing quantitative data on protein expression levels between these two breast cancer subtypes.

- Extracted proteins of the TN and Her2+ group were labeled with fluorescent dyes (Cy3, Cy5, Cy2)
- To achieve better resolution of the protein mixtures the samples were applied to two complementary pH ranges (pH 4-7 and 7-10 on 18 cm IPG strips)
- Gels of each pH range were run in quadruplet
Protein identification

- Spots passing certain statistical criteria with and showing at least a 1.3-fold change in spot intensity were selected for further analysis.
- Gels were silver-stained for spot picking.
- Samples were analyzed by MALDI-TOF mass spectrometry in the positive ion reflector mode.
- Subsequent MS/MS analysis was performed in a data-dependent manner and the eight most abundant ions were subjected to CID (collision-induced dissociation).

**Figure 3:** 3D-view of identified differentially expressed proteins visualized with DeCyder software

- A. CK7 1.84-fold down-regulation
- B. Fibronectin 1.3-fold up-regulation
- C. NME1-NME2 protein 1.49-fold down-regulation
- D. Annexin A1 1.34-fold up-regulation

We identified 13 up-regulated proteins and more than 20 down-regulated proteins in TN breast cancers.
The results obtained from 2D-DIGE and mass spectrometry were validated by Western blot analyses (not shown) and immunohistochemistry (Fig. 4).

**Figure 4** (positive reactions = stained brown)

- Immunohistochemical staining of individual tissue samples
- Confirmation of expression levels of cytokeratins 7 and 14
- CK14 is one of the marker proteins which are used to define the basal-like phenotype of breast cancer to which the majority of TN breast cancers belong to

Cytokeratins are involved in cell motility, cell signaling and cell cycle regulation.
The identified proteins may represent potential therapeutic targets and thus enable effective treatment of TN breast cancer.

The identified proteins are known to be involved in pathways connected to tumor progression and invasiveness:

**Fibronectin**
Is involved in carcinoma development and stimulates the **phosphatidylinositol 3-kinase (PI3K)** pathway

**L-plastin**
Is involved in migration, invasion and metastasis

**NME1- NME2 protein**
Is a key molecule in breast tumor angiogenesis that inhibits the metastatic potential of cancer cells

**Lactoferrin**
Has an effect on **mitogen-activated protein kinase (MAPK)** and the **nuclear factor-kB (NF-kB)** pathways and functions as a regulator of gene expression

**Annexins**
Are involved in signal transduction, mediation of apoptosis, regulation of cytokine production, and in the **MAPK** signal transduction pathway