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Life Sciences, Genomics and Biotechnology for Health

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Coordinator: University of L'Aquila, Italy

MetaBre introduction

MetaBre is a EU-funded research project that aims to identify the underlying mechanisms that cause metastasis in breast cancer. It is a multinational project involving 12 partners, and is supported with € 4m from the EU FP6 programme for 4 years.

There has been considerable success in the treatment of breast cancer in recent years, if detected in its early stages. However, breast cancers are very prone to metastasise, and cause secondary tumours in bone, liver, lungs, brain and lymph nodes. Once solid metastatic tumours are established, the likelihood of complete remission falls, and patients can suffer symptoms generated by metastases that affect quality of life.

Breast cancer statistics

- More than 200,000 women diagnosed in Europe every year
- Lifetime risk of developing breast cancer 1 in 10
- Leading cause of death in women between ages 35 to 55

Source: IARC

Metastasis in breast cancer is a complex multistep process. Genetic changes in tumour cells give rise to aggressive metastatic cells, and these home in on specific organs because of a complex web of molecular and matrix interactions with the organ microenvironment. Understanding the key molecular mechanisms of these metastatic processes can lead to improvements in the prognosis and treatment of breast cancer patients.

MetaBre aims

MetaBre aims to discover new gene and protein markers that can be used for diagnosis as a "signature" of metastasis to specific organs, and also can be targets for therapy.

To achieve this, the partners have analysed samples of breast tumours and metastases, with due care of ethical aspects, as well as established breast cancer cell lines.

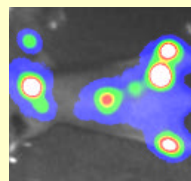
MetaBre is also studying genes and molecules that are already suspected of involvement in metastasis. This builds on previous work of the partners and will enhance understanding of the role of these molecules in metastasis, as well as identifying new therapies and diagnostic methods against these targets.

MetaBre workplan

MetaBre has research activities aimed at:

- Gene profiling and proteomic analysis to identify new molecular targets
- Functional analysis of new targets in in-vitro and in-vivo models
- Mechanisms of angiogenesis and invasion
- Organ-cancer cell interactions
- Development of new pharmacological therapies and diagnostic techniques
- Preliminary clinical trials

MetaBre has used state-of-the-art Affymetrix™ chips for gene profiling and will develop novel in-vitro and in-vivo models for validation of molecular targets and screening of therapeutic molecules.



Metastases will be detected in-vivo with optical imaging of luciferase-expressing cancer cells and magnetic resonance techniques





Progress in year 3

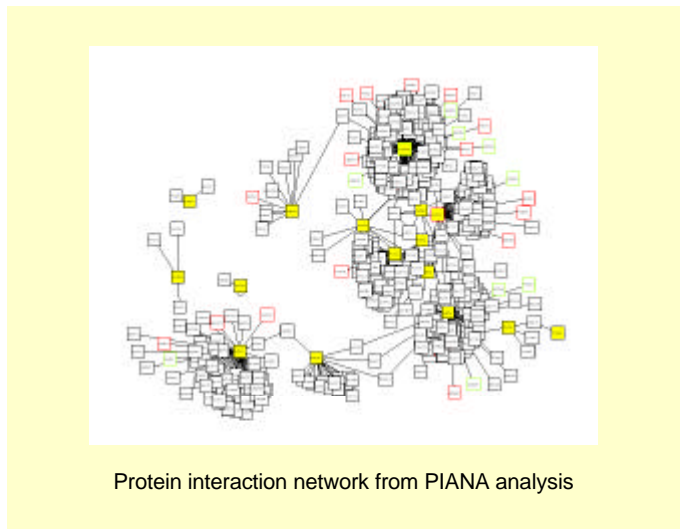
The MetaBre project has now moved into its final phase, and has been granted an extension until December 2007. In year 2, the main work was completed on gene expression and proteomic analysis of cell lines with organ-specific tropisms and tissues of metastases found in different organs. Over 50 genes and proteins of interest were identified for further study, as well as four gene signatures for organ-specific metastasis. Some further gene expression and proteomic analysis has continued, applying new techniques of cell surface proteome analysis and analysing new cell line sub-clones that have been obtained by the partners during the MetaBre project.

Meanwhile, in year 3 the MetaBre consortium has progressed much further in functional analysis of selected genes and proteins, and testing of the gene signatures in a larger collection of human tissue samples. The first set of genes of interest were identified in year 1 from the osteotropic B02 cell line, a sub-clone of breast cancer cell line MDA-MB0-231. Selected genes from this set are most advanced in terms of functional studies. The genes identified in B02 as well as the actual bone metastases samples are implicated in the osteomimicry hypothesis, which suggests that breast cancer cells take on aspects of the phenotype of bone cells in order to thrive in the bone microenvironment.

Functional studies of these osteomimicry genes are being performed by partners UNIVAQ, CMNS, INSERM, PSK, LUMC and ULg. The first step is to check the expression of the encoded protein in bone metastasis tissues, typically paraffin-embedded samples, by immunohistochemistry. Once expression at the protein level is confirmed, breast cancer cell lines are modified in the expression of the gene of interest, through overexpression or gene silencing by siRNA or shRNA.

As an example, for a study of gene S100A4, the B02 cell line has been transfected with recombinant S100A4 in order to obtain a cell model with restored expression of this gene, which is strongly downregulated in B02 compared with the parental MDA-MB-231 cell line. These cell models can then be compared with the original cell lines in in vitro assays, as well as testing for growth of bone metastases in animal models using bioluminescent imaging of the cancer cells. Results from this study should be published in the next year.

The functional studies are complemented by an in silico analysis by IDIBELL (previous name IRO) that uses the PIANA system to interrogate protein databases and identify other proteins interacting with a protein of interest. This can help develop a functional hypothesis for metastatic processes, particular those that assist cancer cells to thrive in the microenvironment of host organs. Also the PIANA analysis has identified linker proteins that interact with many of the proteins identified through gene expression and proteomic analysis, which are interesting for further study.



Functional studies have also continued on molecules that were previously identified as being potentially involved in processes of metastasis in breast cancer. Work on HDAC-regulated genes conducted by ULg and CRH had identified two genes that could be important for breast cancer metastasis. However, further studies on the genes have been hindered by the unavailability of suitable antibodies for their detection. This problem has been overcome in the case of another gene, FGD1, which was identified in the B02 gene expression data. Partner CMNS has succeeded in raising an antibody for this gene and the studies are proceeding in-vitro and in-vivo.

Partner WUELS (previous name WAU) has continued to focus efforts on development of experimental models for studying the role of sialyl Lewis antigens in breast cancer metastasis. Working with LUMC, several breast cancer cell lines with different levels of sialyl Lewis A or X antigens are being modified to obtain "loss of function" and "gain of function" models. As the example, the MDA-





MB-231 and B02 cell sublines, which give metastases to bone, are being transfected with the gene for Fut3 to generate the synthesis of sialyl Lewis antigens in order to obtain perhaps a change in the osteotropic behaviour of these cell lines which have negligible expression of sialyl Lewis. These cell lines have been tested in functional in-vitro shear flow adhesion assays, and in vivo tests at LUMC will commence early in 2007.

Partner ICR has continued to investigate molecular mechanisms of lymph node metastasis. Cell line variants have been obtained in animal models through two different methods (mammary fat pad and intra-vein injection) that represent the putative passive and active mechanisms of metastasis to the lymph nodes. These cell lines are being analysed by microarray to identify a molecular signature for cancer cells which metastasise by each mechanism. Both mechanisms appear to be important, though this is a matter of quite some debate currently among cancer researchers. The cell lines are also being tested for expression of a range of known metastasis molecules, including chemokines and integrins.

UGent has also focused heavily this year on investigating the role of Ncadherin in breast cancer metastasis. This cell surface molecule is strongly upregulated in many cancers. In particular two angiogenesis assays have been used to confirm the pro-angiogenic function of the molecule, and an interaction has been found between N-cadherin and a BMP receptor in-vivo. This could be important for tumour-host interactions, particularly in the bone microenvironment. Also related to angiogenesis, INSERM has commenced a study of the role of VEGF in bone metastasis in breast cancer, which to date has not been studied.

LUMC has an ongoing interest in the dormancy of cancer cells in the bone microenvironment and how the switch to aggressive growth of bone metastases is mediated by molecules such as TGF β and BMP7. Breast cancer cell lines including B02 have been transfected with BMP7 to permit studies of this process in-vivo. These cells have been tested in animal models with bioluminescent imaging and interesting results have been obtained.

Partners CRH, UNIVAQ, IDIBELL and UGent are taking forward the gene signatures and proteomic data to develop new prognostic and diagnostic techniques for clinical application. The four gene signatures for metastasis to bone, brain, liver and lung organs have been tested by CRH for prognostic value in its collection of tissue samples from breast cancer primary tumours, as

well as in gene expression data published from other studies. The results of these tests and the gene signatures themselves will be published in 2007 and a patent is also being registered.

UNIVAQ is also re-examining the gene expression data from the bone metastases and correlating this with actual clinical outcome among the patients who supplied the samples. The hypothesis is that bone metastases develop first, then life-threatening visceral metastases. Gene expression analysis of bone marrow aspirates may indicate which patients will proceed to develop life-threatening visceral metastases and can be targeted for preventative treatment.

IDIBELL has also compared the gene signatures with the results of its proteomic analysis of cell lines to identify proteins that are of interest for prognosis or diagnosis of organ-specific metastasis in breast cancer. A number of proteins related to metastasis to bone, lung and brain have been selected and these will be developed further as markers. Initially this development will be performed by ELISA but IDIBELL are looking eventually to develop antibody arrays with the support of a large Spanish company. UGent have also started to test individual molecules identified in the MetaBre signatures for their potential as serum biomarkers of organ-specific metastasis. This is being performed by ELISA on a collection of blood sera from breast cancer patients, assembled by the partners.

Research also continues to test new pharmacological therapies. While therapies targeting new molecules identified in MetaBre will only be developed after the project, in the meantime other known metastasis molecules have been targeted. PSK is completing its in-vivo screening and testing of integrin receptor antagonists, which were initially developed as a result of studies with the B02 cell line, and is proceeding to early clinical trials.

The MetaBre project was presented at the 5th European Breast Cancer Conference, in Nice, March 2006, at a satellite session hosted jointly with the EU-funded TransBIG project. This is a major conference attended by clinicians working with breast cancer patients from across Europe, and the satellite session was well attended by over 250 people. Furthermore, the MetaBre partners have participated in a conference organised by the "twin" project of MetaBre, BRECOSM. Some collaboration has been organised between the two projects, including a joint publication and conference to be held in Rome, December 2007, at the end of both projects.





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The partners have links with clinicians, patient groups and other related research projects through the MetaBre observer group.

If you are interested to find out more about MetaBre, please consult the project website at www.metabre.org or contact us below:

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