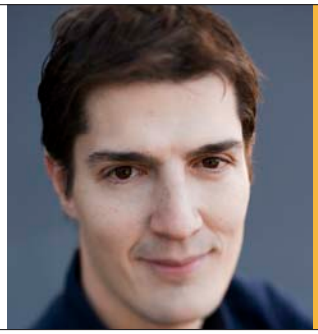
ROGER GOMIS

Tumoral Metastasis Laboratory (MetLab)



Intricate signalling networks control cell division, differentiation, movement, organisation and death. Cancer cells disobey these signals during tumour progression and metastasis, which is the final step in 90% of all fatal solid tumours. Metastasis is therefore a grave public health problem and consequently a field of considerable pharmaceutical interest. A major research focus of our group is to identify and study the genes and functions that allow tumour cells to achieve metastatic colonisation of vital organs.

Growth control and cancer metastasis

Our research focuses on aberrant gene responses that enable invasion and metastasis in tumour cells. We seek to elucidate the mechanisms mediating tissue-specific metastasis, in particular in breast cancer. Metastasis, a complex process caused by elaborate interactions between tumour cells and the surrounding healthy tissues in several vital organs, accounts for 90% of all cancer deaths in patients with solid tumours. The molecular and cellular mechanisms that lead primary tumours to form metastases must be elucidated in order to better address this major life-threatening condition. The identification of metastatic genes and mechanisms is essential to understand the basic biology of this lethal condition and its implications for clinical practice (Fidler, 2003; Gupta and Massagué, 2006). We aim to explain how and why metastasis occurs, the mechanisms that make metastasis a tissue-specific process, the events that allow dormant metastases to become active and lethal many years after removal of a primary tumour and the metastasis-mediating genes that would eventually constitute worthy therapeutic targets.

Our contribution to the field builds on a novel experimental approach based on the use of moderately metastatic cells that are injected into a mouse model for the selection of highly metastatic breast cancer subpopulations. Live animal-imaging techniques are used to track the spread, homing, and outgrowth of the metastatic cells in several organs. After harvesting metastatic lesions and verifying that highly metastatic cells have been selected, we use genome-wide transcriptomic profiling to identify metastasis-linked genes. Gene transfer techniques are then used to assess the contribution of individual genes to various steps (invasion, homing, outgrowth, angiogenesis, and stroma adaptation) of the metastatic process.

Breast cancer is the most frequently diagnosed cancer in women in Europe and the United States, with an estimated 608,380 new cases of invasive disease in 2007 (American Cancer Society, 2007; Ferlay *et al*, 2007). Despite a recent decrease in its incidence rates in Europe and the United States (Ravdin *et al*, 2007), it remains the second leading cause of cancer deaths among women. Most of these patients die as a result of the metastatic spread of the tumour. Our current understanding of the biology of breast cancer is a major barrier to identify novel therapies and improve existing therapies for the treatment and prevention of this disease.

The predisposition of primary tumours to selectively invade different organs has long been recognised (Paget, 1889). Recent work has functionally identified and clinically validated sets of genes whose overexpression in estrogen receptor (ER)-negative breast cancer and prostate cells confers a selective advantage for the colonisation of bones (Kang *et al*, 2003; Lynch *et al*, 2005) and lungs (Minn *et al*, 2005). Moreover, under certain conditions tumour cells cannot grow or survive in the absence of a supportive microenvironment. Indeed, the microenvironment may even drive tumour and metastasis development by selecting for highly invasive and resistant cancer cell phenotypes (Bernards and Weinberg, 2002) and systemically fostering the mobilisation of marrow-derived progenitor cells (Kaplan *et al*, 2005). The capacity to subsequently colonise distant organs depends on the organ-colonising faculties of disseminated tumour cells as well as on certain requirements that may be present in the otherwise restrictive microenvironment of target organs (Gupta and Massagué, 2006). Thus, the various steps of metastasis do not necessarily represent the acquisition of individual specialised mutations but rather the random accumulation of traits that provide the advantage necessary to adapt to the microenvironment of a given organ.

Breast cancer is a remarkably heterogeneous disease, but subsets of tumours show recurrent patterns of transcriptional, genomic, and biological abnormality. Understanding how genes in

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these 'patterns' collectively function in an otherwise heterogeneous biological setting to enable progression and modulate response to therapy is critical to improve management of the disease. In particular, we aim to determine how the ER and HER2 pathways contribute to leading molecular events in breast cancer metastasis. ERα is overexpressed in around 65% of breast cancer cases, referred to as 'ER-positive'. Binding of estrogen to the ER stimulates the proliferation of mammary cells. ER-positive tumour cells are highly dependent on this stimulus to proliferate, and therefore ER is currently used as a therapeutic target (Ali and Coombes, 2002). Approximately 15-20% of breast cancers have an amplification of the *HER2* gene or overexpression of its protein product. HER2 is a cell membrane receptor tyrosine kinase and is normally involved in the epidermal growth factor signal transduction pathway leading to cell growth and proliferation. Overexpression of this receptor in breast cancer is associated with increased disease recurrence and poor prognosis (Slamon *et al*, 2001).

ER- and HER2-positive breast cancer cells preserve, among each subtype, genome-aberration-induced transcriptional changes with high fidelity. The resulting dominant genes will reveal molecular events that predict the metastatic outcome despite substantial genomic, transcriptional, translational, and biological heterogeneity in the overall system. The two tumour subtypes may metastasise to the same secondary organ. However, it is unknown whether the developmental history of a cancer would result in different or common mediators of site-specific metastasis. Predisposing factors related to the cell of origin may engender several rate-limiting barriers during the progression of metastasis. Our work aims to set the stage for a detailed study of mechanisms of metastasis and their potential value as new therapeutic targets. We are screening metastatic cell populations from pleural effusions derived from breast cancer patients in order to identify new metastatic gene signatures. For this purpose, on the basis of collaborations with clinical and basic researchers at the Hospital Clínic, in Barcelona, and the Memorial Sloan-Kettering Cancer Center, in New York, the MetLab team has initiated the isolation of metastatic cells from pleural effusions derived from lung and breast cancer patients. Once injected in mice, these cells are labelled with the GFP-Luciferase-TK protein fusion and visualised by bioluminescent techniques. On the basis of these metastatic cell populations, highly aggressive subpopulations with tropism to specific tissues will be isolated. These subpopulations will be used to identify and validate metastatic gene signatures by means of gene expression profile analyses and biochemical, cellular and molecular biology techniques.

We have also focused our attention on groups of genes that drive metastatic ER-negative cancer cells to one tissue or another. Particularly, we address metastatic suppressor genes and their functions in the metastatic process. We are conducting studies on the group of metastatic suppressor genes required for breast to lung metastasis, identified in Joan Massagué's laboratory (Minn *et al*, 2005) at the Memorial Sloan-Kettering Cancer Center. For this purpose, we are using the MDA-MB-231 breast cancer cell line model and its derivatives #4175 and #1833, which have a strong metastatic capacity to lung and bone.

Finally, our research centres, in part, on recent progress in the analysis of the TGF-beta cytostatic programme and its evasion in metastatic breast cancer. This project seeks to clarify the role of C/EBPβ transcription factor in the TGF-beta cytostatic programme in epithelial cells. Breast cancer cells are refractory to TGFβ-mediated growth arrest, thus leading to further tumour progression and metastasis. The molecular characterisation of TGFβ-mediated cytostasis in keratinocytes has placed C/EBPβ at the heart of this response. Furthermore, deregulation of C/EBPβ mediates evasion of the TGFβ-induced cytostatic effects in metastatic breast cancer cells. We found that the transcription factor C/EBPβ is essential for not only the induction of the cell cycle inhibitor *p15INK4b* by a FoxO-Smad complex but also for the repression of c-MYC by an E2F4/5-Smad complex. Interestingly, the *p15INK4b* and *c-MYC* gene responses, which are central to the TGFβ cytostatic programme, were selectively missing in primary metastatic breast cancer cells from half of the patients with advanced-stage disease that we analysed. Remarkably, this loss coincided with increased expression of the C/EBPβ inhibitory isoform LIP, which has been implicated in tumour progression. By normalising the LIP:LAP ratio, we restored these TGFβ cytostatic gene responses and growth inhibition in primary metastatic cells derived from human patients. Building on this work, we will determine the mechanism by which LIP expression is deregulated in metastatic breast cancer cells. Thus, by using biochemical and molecular biology techniques, primary human breast cancer cell cultures and animal model studies, we will study the molecular mechanisms that lead to the deregulation of the C/EBPβ function and consequent loss of the TGF-beta cytostatic response in cancer cells.

SCIENTIFIC OUTPUT

Publications

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Research networks and grants

Papel de C/EBPbeta en los mecanismos moleculares de regulación de la respuesta citostática al TGF-beta; implicaciones fisiológicas y sus alteraciones en el cáncer de mama
Spanish Ministry of Science and Innovation, SAF2007-62691 (2007-2009)
Principal investigator: Roger Gomis

Study of the molecular mechanisms of metastasis of breast cancer to the lung: therapeutic function and potential of metastasis suppressor genes
AECC-Spanish Cancer Association (2008-2010)
Principal investigator: Roger Gomis

Other funding sources

Mechanisms of metastasis
BBVA Foundation

Collaborations

Cristina Nadal, Oncology Service, Hospital Clínic Barcelona (Barcelona, Spain)

Awards

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