

# Tumoural Metastasis Laboratory (Metlab)

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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.

Our research focuses on the growth factors, signalling pathways, and gene expression programmes underlying cancer cell metastasis. We study the ways in which cancer cells evade tumour suppressor mechanisms and engage in metastatic behaviour. Focusing on a TGF- $\beta$  cytotostatic programme involving the transcriptional regulation of cell cycle inhibitors and growth-promoting factors, we are investigating how tumour cells evade these gene responses in order to pursue metastatic behaviour. By combining *in vivo* selection of human metastatic cells, transcriptomic profiling and functional testing, we identify genes that selectively mediate breast metastasis to specific organs. Gene transfer techniques and RNAi-mediated gene silencing are used to functionally validate candidate genes. We are encouraged by the recent validation of these findings in clinical samples. Several of these genes encode products that are susceptible to therapeutic targeting.

The Tumour Metastasis Laboratory (Metlab), part of IRB Barcelona's Oncology Programme focuses on the molecular mechanisms involved in metastasis. The activities in this research group began in June 2006.

Current research builds, in part, on recent progress in the analysis of the TGF- $\beta$  cytotostatic programme and its evasion in metastatic breast cancer. This line of research seeks to clarify the role of C/EBP $\beta$  transcription factor in the TGF- $\beta$  cytotostatic programme in epithelial cells. Recent results have provided a new approach to study the molecular mechanisms that regulate this programme. The TGF- $\beta$  signalling process is based on the formation of a TGF- $\beta$ -activated receptor complex that phosphorylates SMAD transcription factors, which in turn assemble molecular complexes that regulate the expression of target genes. Several of these gene responses act in concert to cause cell cycle arrest. This TGF- $\beta$  cytotostatic programme includes repression of the proliferation-promoting genes c-MYC and lcl1, as well as induction of the cyclin-dependent kinase (CDK) inhibitors p15INK4b and p21CIP1 (Massagué and Gomis, 2006). Repression of c-MYC and lcl1 is mediated by a complex of SMAD with E2F4/5 and ATF3, respectively.

FoxO factors were identified as partners of TGF- $\beta$ -activated SMAD3 in the induction of the CDK inhibitor,

p21CIP1, in epithelial cells. In recent work, we have taken a genetic approach to identify other TGF- $\beta$  target genes that are regulated by a common SMAD3/FoxO transcription complex. By using siRNA techniques coupled with gene expression microarray data analysis, 10 new genes whose TGF- $\beta$  expression is induced by the same complex were identified (Gomis *et al*, 2006a). *p15INK4b* (Gomis *et al*, 2006a) stands out among these genes. Surprisingly, a detailed analysis of the *p15INK4b* promoter led to the identification of the role of C/EBP $\beta$  in *p15INK4b* induction by TGF- $\beta$  (Gomis *et al*, 2006b).

Breast cancer cells are refractory to TGF- $\beta$ -mediated growth arrest, which leads to further tumour progression and metastasis. The molecular characterisation of TGF- $\beta$ -mediated cytotostasis in keratinocytes has positioned C/EBP $\beta$  at the core of this response. Furthermore, deregulation of C/EBP $\beta$  mediates evasion of the TGF- $\beta$ -induced cytotostatic effects in metastatic breast cancer cells. We found that the transcription factor C/EBP $\beta$  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 TGF- $\beta$  cytotostatic pro-

gramme, are selectively missing in primary metastatic breast cancer cells from half of the patients with advanced-stage disease that we analysed. Remarkably, this loss coincides with increased expression of the C/EBP $\beta$  inhibitory isoform LIP, which has been implicated in tumour progression. By normalising the LIP:LAP ratio, we restored these TGF $\beta$  cytostatic gene responses and growth inhibition in primary metastatic cells derived from patients. Building on this work, we will determine the mechanism by which LIP expression is deregulated in metastatic breast cancer cells.

Thus, using biochemical and molecular biology techniques, primary human breast cancer cell cultures and animal model studies, we examine the molecular mechanisms that lead to the deregulation of the C/EBP $\beta$  function and consequent loss of the TGF- $\beta$  cytostatic response in cancer cells. In addition, our lab activities focus on extending these findings to other cell types in which the TGF- $\beta$  cytostatic response is either permanently or temporally absent.

The second research project initiated in our lab seeks to identify gene groups that drive metastatic cells to one tissue or another. Particularly, we focus on metastatic suppressor genes and their functions in

the metastatic process. Our initial studies are devoted to the group of metastatic suppressor genes required for breast to lung metastasis, identified in Dr. Joan Massagué's lab (Minn *et al*, 2005). For this purpose, we use 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. Furthermore, we are also screening new metastatic cell populations from pleural effusions derived from breast or lung cancer patients to identify new metastatic gene signatures. For this purpose, on the basis of collaborations with clinical and basic investigators 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. These cells are labelled with the GFP-Luciferase-TK protein fusion and, once injected in mice, are visualised by bioluminescent techniques. From 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.

**PUBLICATIONS**

Gomis RR, Alarcon C, He W, Wang Q, Seoane J, Lash A and Massagué J (2006) A FoxO-Smad synexpression group in human keratinocytes. *Proc Natl Acad Sci USA*, 103:12747-12752

Gomis RR, Alarcon C, Nadal C, Van Poznak C and Massagué J (2006) C/EBP $\beta$  at the core of the TGF $\beta$  cytostatic response and its evasion in metastatic breast cancer cells. *Cancer Cell*, 10:203-214

Massagué J and Gomis RR (2006) The logic of TGF $\beta$  signalling. *FEBS Lett*, 580:2811-2820

**OTHER FUNDING**

*Mechanisms of metastasis*  
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**COLLABORATIONS**

*Identification of genes involved in breast and lung cancer metastasis*  
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