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 colonization of vital organs.

**Growth control and cancer metastasis**

Our research centres 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 cytostatic programme involving the transcriptional regulation of cell cycle inhibitors and growth-promoting factors, we are examining 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 seek to identify genes that selectively mediate breast metastasis to specific organs. Gene transfer techniques and RNAi-mediated gene silencing are used to functionally corroborate 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.

Current research builds, 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/EBP transcription factor in the TGF-beta cytostatic programme in epithelial cells. Recent results have provided a new approach to explain the molecular mechanisms that control 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 cytostatic programme includes repression of the proliferation-promoting genes *c-MYC* and *ld1*, as well as induction the cyclin-dependent kinase (CDK) inhibitors p15INK4b and p21CIP1 (Padua et al, 2008). Repression of *c-MYC* and *ld1* 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, ten new genes whose TGF-beta expression is induced by the same complex were identified (Gupta et al, 2007). p15INK4b stands out among these genes. Surprisingly, a detailed analysis of the p15INK4b promoter has led to the finding of a role for C/EBP-beta in p15INK4b induction by TGF-beta (Gomis et al, 2006b).
Breast cancer cells are refractory to TGF-beta-mediated growth arrest, thus leading to further tumour progression and metastasis. The molecular characterisation of TGF-beta-mediated cytostasis 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 cytostatic 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 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/EBP-beta inhibitory isoform LIP, which has been implicated in tumour progression. By normalizing the LIP:LAP ratio, we restored these TGF-beta 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/EBP-beta function and consequent loss of the TGF-beta cytostatic response in cancer cells. In addition, our laboratory focuses on extending these findings to other cell types in which the TGF-beta cytostatic response is absent either permanently or temporally.

The second research project started in our laboratory aims 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 research into 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. Furthermore, we are also screening new metastatic cell populations from pleural effusions derived from breast or lung cancer patients in order to identify new metastatic gene signatures. For this purpose, on the basis of collaborations with clinical and basic investigators at the Hospital Clinic, 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 sub-populations 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**


**Other references**


**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**


Research Director: Roger Gomis

**Study of the molecular mechanisms of metastasis of breast cancer to the lung: therapeutic function and potential of metastasis suppressor genes**

Asociación Española Contra el Cáncer (Madrid, Spain)

Research Director: Roger Gomis

**Other Funding Sources**

**Mechanisms of metastasis**

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**Collaborations**

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