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METASTASIS

Recharging with COCO

Available evidence mostly supports the idea that, in many tumour types, tumour cells disseminate to metastatic sites early in tumour progression and then often lie dormant for an extended period before becoming reactivated to produce overt metastases. Filippo Giancotti and colleagues have uncovered a possible molecular mechanism controlling this reactivation of disseminated breast cancer cells.

The authors conducted a gain-of-function screen by expressing a cDNA library derived from lung metastatic 4T1 mouse mammary carcinoma cell lines in 4TO7 cells, which disseminate but which do not produce macrometastases. They isolated three cDNAs that promoted lung metastasis following mammary fat pad injection of 4T07 cells without affecting primary tumour proliferation. One of these encoded a biologically functional fragment of COCO (also known as DAND5), a secreted inhibitor of transforming growth factor-β (TGFβ) family members, including bone morphogenetic proteins (BMPs). Expression of full-length COCO also promoted lung colonization of 4TO7 cells after orthotopic injection into syngeneic mice. In addition, COCO did not affect primary tumour growth or enhance invasive properties of the cells, but permitted colonization of the lungs (as shown by tail-vein injections). Confocal imaging further showed that COCO did not change the ability of 4TO7 cells to infiltrate the lungs, but specifically promoted metastatic outgrowth, suggesting that it allows reactivation of the cells from dormancy. Conversely, silencing of COCO in the metastatic 4T1 cells reduced metastasis and kept disseminated cells in the lung quiescent.

> “[COCO] allows reactivation of the cells from dormancy”

Lara Crow/NPG

How might COCO promote metastatic outgrowth? COCO seemed to block activation of BMP signalling, as shown by reduced SMAD phosphorylation (P-SMAD) following BMP4 stimulation of 4TO7 cells *in vitro*. Furthermore, the lung microenvironment contained high levels of several BMPs, and most dormant 4TO7 cells in the lungs had high levels of P-SMAD. However, a small number of these, as well as the macrometastases, had no nuclear P-SMAD, suggesting that cells that suppress BMP signalling may give rise to overt metastases. Conversely, silencing COCO in 4T1 cells reactivated SMAD signalling and enforced dormancy. In addition, inhibition of BMP signalling by expressing a dominant-negative BMP receptor (BMPR) permitted lung colonization by 4TO7 cells and COCO-silenced 4T1 cells, and expression of an active BMPR in 4T1 cells blocked lung colonization.

Previous studies have suggested that metastasis-initiating cells might share some properties with cancer stem cells (CSCs), so the authors examined the effects of COCO and BMP signalling on CSC-like traits. Exogenous BMP4 blocked the formation of mammospheres (an *in vitro* measure of self-renewal) by 4TO7 cells, and this was reversed by treatment with exogenous COCO. COCO expression also increased the expression of several stem cell transcription factors in 4TO7 cells, and this was suppressed by treatment with BMP4. 4T1 cells expressing an active BMPR also had reduced tumour-initiating capacity when injected into mammary fat pads of syngeneic mice.

Is this pathway relevant to human breast cancer? MDA-MB-231 human breast cancer cells, which can colonize the lungs of nude mice, had high levels of COCO expression, and COCO silencing suppressed lung colonization and CSC-like properties of these cells. A 14-gene expression signature induced by COCO expression was predictive of overall metastatic relapse in several cohorts of patients with breast cancer, and two genes alone (*KIAA1199* and *NDRG1*) predicted relapse with a similar efficiency. Further analyses indicated that the 14-gene and two-gene signatures selectively predicted relapse to the lung, but not to the brain or bone. Lung-specific metastasis by COCO-expressing cells and low levels of BMP expression in the brain and bone were confirmed using mouse models.

Overall, these data suggest that dormant cancer cells that ultimately give rise to metastases need to overcome organ-specific suppressive signals in the microenvironment, and that in doing so, they may induce CSC-like functions.

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ORIGINAL RESEARCH PAPER Gao, H. *et al.* The BMP inhibitor Coco reactivates breast cancer cells at lung metastatic sites. *Cell* **150**, 764–779 (2012)