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## NEWS

# Cancer stem cells and tumorigenesis

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Recent *IBMS BoneKEy* webinar stressed importance of intrinsic CSC properties and interactions with the tumor microenvironment.

What gives cancer stem cells (CSCs) their unique ability to initiate tumors? This question was at the heart of Putting Cancer Stem Cells into Context, a recent *IBMS BoneKEy* webinar presented by Ilaria Malanchi (London Research Institute, United Kingdom). The message that Dr Malanchi delivered was that, although CSCs have inherent properties that endow them with their tumorigenic potential, interactions that the cells have with their surrounding milieu, in a primary tumor or at a metastatic site, are just as crucial. 'It is indeed important that CSCs have intrinsic growth and self-renewal abilities,' Dr Malanchi said. 'But successful crosstalk between CSCs and their environment is also important for tumor initiation.'

After Dr Malanchi's talk, a distinguished panel including Dominique Bonnet (London Research Institute, United Kingdom) and Gabri van der Pluijm (Leiden University Medical Center, The Netherlands) participated in a discussion moderated by Philippe Clézardin (INSERM, Lyon, France), *IBMS BoneKEy* Associate Editor. The webinar is available for viewing at: http://www.nature.com/bonekey/webinars/index.html?key=webinar29.

Dr Malanchi opened her talk with the observation that not all cancer cells are the same, as is reflected in differences amongst those cells in their self-renewal and growth properties and their capacity to initiate tumors. Amongst the cancer cell population, CSCs have the highest tumorigenic potential and are responsible for tumor homeostasis. If that is so, then eliminating CSCs should cause tumors to regress.

In the first part of her presentation, Dr Malanchi discussed her work, published in *Nature* in 2008, which put that hypothesis to the test using a chemically induced skin tumor model in mice.[1] She and her colleagues first found that skin CSCs that expressed CD34, a marker that normal skin stem cells also express, were much more efficient at generating tumors in a skin transplantation assay compared with when all tumor cells were transplanted. 'When we transplanted only the $CD34^+$ cells, we saw a shift in tumor-generating efficiency of roughly 100-fold,' she said. They also found that $CD34^-$ cells never generated tumors.

The next step was to understand the mechanism by which CSCs were regulated in the tumors. Using transgenic mice that had a reporter gene knocked into a locus known to be a target gene of Wnt signaling, the investigators found that Wnt signaling was present in tumors but not in normal skin. On the basis of that finding, the team used another mouse model to delete *β-catenin*, a gene in the Wnt signaling pathway, from tumors. Deleting the gene from established skin tumors resulted in complete tumor regression, whereas control tumors continued to grow. Further analysis indicated that depletion of *β-catenin* reduced the numbers of CSCs, and that transplanted tumor cells missing *β-catenin* were never able to generate secondary tumors, regardless of the number of transplanted cells. These results provided evidence of a causal chain whereby loss of CSCs resulted in loss of tumor initiation potential and then loss of tumor homeostasis.

Dr Malanchi then turned to more recent work, also published in *Nature*, examining the role of CSCs in the metastatic process.[2] During metastasis, only a very small percentage of cells that successfully extravasate from the circulation into a metastatic site are able to persist there and form micrometastases, and an even smaller percentage of those cells are able to establish macroscopic tumors. The starting point of Dr Malanchi's research in this area was to investigate whether the hierarchy observed in primary tumors, in which CSCs have the highest tumorigenic potential, also characterizes secondary sites and explains metastatic success.

To explore that question, Dr Malanchi and her colleagues used a mouse breast cancer model characterized by spontaneous lung metastasis. They first found that cells positive for two previously established CSC markers, CD90 and CD24, initiated lung metastases when injected into the tail vein of the animals, whereas $CD90^+CD24^+$-depleted cell populations were unable to do so. They further learned that, just as in the primary tumor site, the frequency of $CD90^+CD24^+$ CSCs at the metastatic site was just 2%, revealing that the hierarchy of the primary tumor was indeed preserved in the metastasis.

The researchers next examined the time course of metastasis to better understand the role of CSCs during the metastatic process. After injecting all tumor cells from the parent tumor into the lung, the team observed that the relative amount of CSCs among all tumor cells in the lung increased to more than 20% within the first week after injection, as a result of CSC proliferation. Meanwhile, the proliferation of non-CSCs decreased during the first week, and those cells could not give rise to CSCs. These results indicated that CSCs were responsible for early metastatic colonization.

However, because only a very small proportion of CSCs that give rise to micrometastases give rise to macroscopic tumors, CSCs cannot be the only factor explaining metastatic success, suggesting that the local microenvironment, known as the tumor niche, that surrounds CSCs is important in this regard, just as a stem cell niche is important for the maintenance of normal stem cells in tissues. One goal of Dr Malanchi's work was to pinpoint signals coming from the tumor niche that allow CSCs to give rise to metastases. Tumor gene expression analyses led Dr Malanchi and her co-investigators to *Postn*, which encodes an extracellular matrix protein, periostin.

Could periostin be a key CSC niche signal? Using their mouse breast cancer model, the investigators discovered that stromal cells surrounding primary breast cancer tumors, and stromal cells surrounding breast cancer micrometastases in the lung, expressed periostin. To further elucidate a role for periostin, knockout mice missing the protein were generated and crossed with the mouse breast cancer model. Although there were no differences in primary tumor size or number between control animals and periostin knockout mice, lung metastases in the periostin knockout mice were almost completely eliminated. Further experiments would show that lung metastasis required periostin expression in the stroma and that periostin was necessary for the maintenance of CSCs.

Subsequent experiments were designed to determine how periostin functioned to maintain CSCs. A tandem mass spectometry analysis would reveal that periostin interacted with a Wnt ligand, and experiments would confirm binding of periostin to Wnt3a and Wnt1 *in vitro*. Furthermore, the presence of periostin augmented Wnt signaling, as revealed by an *in vitro* Wnt reporter assay. The researchers were also able to demonstrate the importance of Wnt signaling in CSCs *in vivo*, in the setting of metastasis, using cancer cells expressing a Wnt signaling reporter gene. They found that CSCs in lung metastases showed much greater Wnt signaling activity compared with non-CSCs, and that CSCs exhibited Wnt signaling activity only when periostin was present. Furthermore, breast cancer cells with endogenously driven Wnt signaling activity could form lung metastases even in periostin knockout mice.

Together, the results from the periostin and Wnt signaling experiments revealed the importance of crosstalk between CSCs and components of the CSC niche for metastatic growth. In this scenario, CSCs release molecules—one of which, Dr Malanchi has shown, is transforming growth factor (TGF)-$\beta$—that increase the expression of periostin by stromal cells surrounding CSCs. In turn, the binding of periostin to Wnt ligands keeps those ligands close to CSCs, allowing for increased Wnt signaling into CSCs, which increases the likelihood of metastases.

Ultimately, it is not only the capacity of CSCs to grow and self-renew, but also their ability to exploit their surrounding environment, which endows these cells with their tumorigenic potential. In terms of capitalizing upon this knowledge for therapeutic purposes, Dr Malanchi noted that because CSCs and normal stem cells have many similarities, targeting interactions between CSCs and their environment may be a more fruitful approach toward developing new cancer treatments, rather than targeting CSCs directly.

## Conflict of Interest

The author declares no conflict of interest.

## References

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