

Break the loop, escape the cycle?

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Increasing evidence suggests that in many cancer types only a minor proportion of cells, the so-called ‘cancer stem cells’, is responsible for fostering continuous tumour growth. Similar to the non-malignant stem cells that maintain tissue homeostasis, cancer stem cells are seemingly able to self-renew indefinitely. A recent study from the lab of Walter Birchmeier, in cooperation with Ulrike Ziebold, published in *The EMBO Journal* (Wend *et al.*, 2013) suggests that cancer stem cells hijack self-renewal mechanisms similar to those observed in (induced) pluripotent stem cells. Interestingly, their data indicate that breaking this self-enforcing, proliferative loop might be sufficient to promote cancer stem cell differentiation and exhaust tumour growth.

Upon analysing a series of salivary and head and neck squamous cell carcinomas the authors observed that these tumours often expressed high levels of nuclear β -catenin while lacking nuclear phospho-Smad staining, signifying augmented and reduced signalling through the WNT and BMP pathways, respectively. This prompted the authors to assess the role of these two signalling pathways in tumour formation by generating a conditional mouse model that allows the concomitant activation of Wnt/ β -catenin (using a dominant-active *Ctnnb1*^{GOF} allele) and inactivation of BMP signalling (homozygous *Bmpr1a*^{LOF}) in keratin K14 (*K14*^{Cre})-expressing epithelial cells. This resulted in the efficient formation of murine salivary tumours that closely resembled high-grade human salivary gland cancers.

A powerful method to identify cancer stem cells is to show their ‘tumour initiating’ or ‘tumor propagating’ capacity in transplantation experiments (Al-Hajj *et al.*, 2003). The authors were indeed able to isolate such cells from the murine *Bmpr1a*^{LOF};*Ctnnb1*^{GOF} salivary gland tumours. Cells with a CD24⁺;CD29⁺ marker profile appeared far more efficient in forming tumours upon (serial) transplantation into immunodeficient recipient mice than unsorted tumour cells. Moreover, upon transplantation these CD24⁺;CD29⁺ tumour-propagating cells restored the CD24;CD29 profile of the original tumour, with ~10% of the tumour cells being CD24⁺;CD29⁺. This highlights a second important hallmark of cancer stem cells: they re-establish the cellular hierarchy of the tumours from which they were isolated, including the self-renewing cancer stem cell compartment. So then what drives this process?

Part of the answer came from transcriptional profiling and gene-set enrichment analyses. Compared to non-transformed CD24⁺;CD29⁺ cells, the cancerous CD24⁺;CD29⁺ population showed enhanced expression of several pluripotency genes. In addition, these cells also displayed increased H3K4me3 (a mark for transcriptionally active promoters) and reduced H3K27me3 (associated with silent promoters) levels. Taken together, these analyses suggested that the

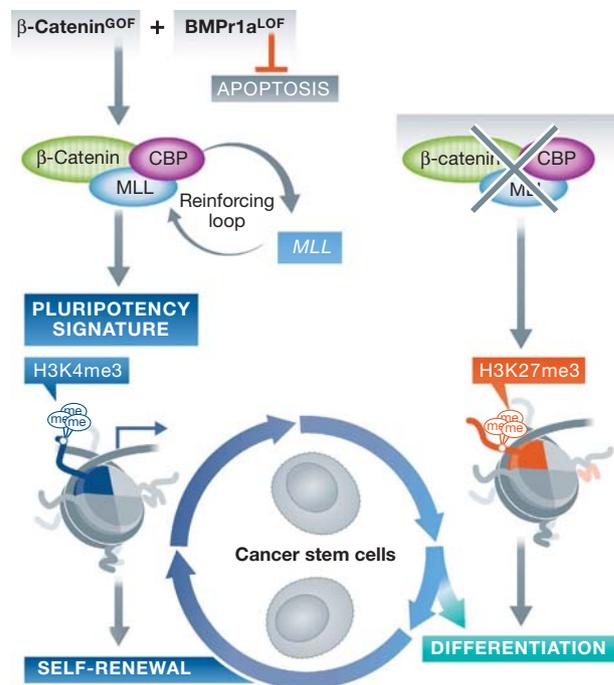


Figure 1 Squamous cell carcinomas of the salivary gland induced by the combined gain of Wnt/ β -catenin and loss of BMP-signalling contain a population of rapidly cycling cancer stem cells. Loss of BMP signalling serves to prevent cells from undergoing apoptosis. The epigenetic state of these cancer stem cells has become rewired to induce the expression of a pluripotency gene network, owing to the activity of a β -catenin/CBP/MLL chromatin-modifying complex that maintains an open chromatin structure at target gene promoters by promoting H3K4me3 methylation. Activity of this complex is reinforced by a feed-forward mechanism, as it also induces gene expression of one of its own components, *MLL*. A genetic or pharmaceutical block of the β -catenin/CBP/MLL complex restores the epigenetic rewiring, resulting in increased H3K27me3 methylation, loss of self-renewal capacity and differentiation of the cancer stem cell population.

overall epigenetic signature of the *Bmpr1a*^{LOF};*Ctnnb1*^{GOF} cancer stem cells had changed profoundly, resulting in the re-expression of a gene network that is normally associated with the embryonic or induced pluripotent stem cell state (Figure 1).

Given that the cancer stem cell population is presumed to be responsible for fuelling tumour growth, it is thought to be critical to specifically target this cell population, both to exhaust tumour growth and to prevent relapse (Yu *et al.*, 2012). Unfortunately, these cancer stem cells may be the most refractory to treatment due to increased resistance to currently available chemotherapeutic agents and radiotherapy (Malik and Nie, 2012). This underscores the need for novel intervention strategies. It is promising, therefore, that Wend *et al.* (2013) were able to stall the growth of cancer stem cells by intervening with the Wnt/ β -catenin pathway, either by knocking down *Ctnnb1* expression or by treating the cells with ICG-001, a small-molecule inhibitor that disrupts the association between β -catenin and its co-factor CBP, a chromatin remodelling protein. Inhibition of Wnt/ β -catenin signalling reverted both the expression of pluripotency genes and the changes in the histone code. In otherwise rapidly expanding salisphere cultures, this also halted self-renewal and induced differentiation of *Bmpr1a*^{LOF};*Ctnnb1*^{GOF} cancer stem cells. This again required epigenetic rewiring, as both the DNA methyltransferase inhibitor 5-azacytidine and the HDAC inhibitor valproic acid prevented differentiation in the presence of ICG-001.

Interestingly, the authors identify the histone methyltransferase and known β -catenin binding partner MLL as a crucial player in the epigenetic rewiring of the cancer stem cell population. Similar to treatment with ICG-001 or knocking down *Ctnnb1*, genetic knockdown of *MLL* expression reduced the number of H3K4me3-positive cells, inhibited cell proliferation and promoted differentiation of cancer stem cells *in vitro*. Of note, *MLL* was also part of the pluripotency gene signature detected in the CD24⁺;CD29⁺ cancer stem cell population. This latter observation suggests the existence of a reinforcing feed-forward loop, comparable to that observed in embryonic stem cells, in which transcription factors such as Oct4, Sox2 and Nanog are both targets and critical components of the core complex of pluripotency factors (Boyer *et al.*, 2005; Kim *et al.*, 2008).

So is there any promise for clinical application of these discoveries? The fact that high Wnt/ β -catenin and low BMP

signalling activities are found in high-grade human salivary gland tumours and other squamous cell carcinomas raises the expectation that a similar blockade might work to treat human tumours, although cancer stem cell plasticity may pose an obstacle to irreversible differentiation. In itself, the idea of differentiation therapy is not novel. In fact, it forms the basis for the successful use of *all-trans* retinoic acid (together with chemotherapy or arsenic trioxide) in the successful treatment of acute pro-myelocytic leukaemia (de The and Chen, 2010). While Birchmeier and colleagues show that treatment with ICG-001 did stall growth of grafted CD24⁺;CD29⁺ *Bmpr1a*^{LOF};*Ctnnb1*^{GOF} tumour cells, their paper does not include data indicating that ICG-001 induces differentiation of the cancer stem cell population *in vivo*, similar to the powerful effect observed in salisphere cultures. In addition, mice were not treated with ICG-001 for prolonged periods of time to study an eventual relapse.

On the one hand, the finding that mice could be treated with ICG-001 for a 20-day period without any apparent adverse effects on rapidly proliferating tissues such as the intestine, which critically requires Wnt/ β -catenin signalling for its maintenance, may seem surprising. However, it is in line with earlier studies (Emami *et al.*, 2004) and offers hope for the use of ICG-001 and other recently developed Wnt-pathway inhibitors in a clinical setting (Gurney *et al.*, 2012; Lum and Clevers, 2012; Verkaar *et al.*, 2012). At the same time, however, a report pointing to a detrimental effect of Wnt-pathway inhibition on hippocampal neurons and behaviour (Kim *et al.*, 2011) urges for a thorough evaluation of the potential toxicity of Wnt-signalling inhibitors.

In summary, the finding that cancer stem cells abuse the pluripotency networks that normally maintain the pluripotent stem cell state, underscores the intimate links between stem cell biology and cancer research. It also suggests that rather than actively trying to wipe out the cancer stem cell population, we should consider the possibility of promoting its differentiation by breaking the self-renewal loop. The study by Wend and colleagues demonstrates that this is feasible, provided that the crucial nodes are targeted.

Conflict of interest

The authors declare that they have no conflict of interest.

References

- Al-Hajj M, Wicha MS, Benito-Hernandez A, Morrison SJ, Clarke MF (2003) Prospective identification of tumorigenic breast cancer cells. *Proc Natl Acad Sci USA* **100**: 3983–3988
- Boyer LA, Lee TI, Cole MF, Johnstone SE, Levine SS, Zucker JP, Guenther MG, Kumar RM, Murray HL, Jenner RG, Gifford DK, Melton DA, Jaenisch R, Young RA (2005) Core transcriptional regulatory circuitry in human embryonic stem cells. *Cell* **122**: 947–956
- de The H, Chen Z (2010) Acute promyelocytic leukaemia: novel insights into the mechanisms of cure. *Nat Rev Cancer* **10**: 775–783
- Emami KH, Nguyen C, Ma H, Kim DH, Jeong KW, Eguchi M, Moon RT, Teo JL, Kim HY, Moon SH, Ha JR, Kahn M (2004) A small molecule inhibitor of beta-catenin/CREB-binding protein transcription [corrected]. *Proc Natl Acad Sci USA* **101**: 12682–12687
- Gurney A, Axelrod F, Bond CJ, Cain J, Chartier C, Donigan L, Fischer M, Chaudhari A, Ji M, Kapoun AM, Lam A, Lazetic S, Ma S, Mitra S, Park IK, Pickell K, Sato A, Satyal S, Stroud M, Tran H *et al.* (2012) Wnt pathway inhibition via the targeting of Frizzled receptors results in decreased growth and tumorigenicity of human tumors. *Proc Natl Acad Sci USA* **109**: 11717–11722
- Kim H, Won S, Hwang DY, Lee JS, Kim M, Kim R, Kim W, Cha B, Kim T, Kim D, Costantini F, Jho EH (2011) Downregulation of Wnt/beta-catenin signaling causes degeneration of hippocampal neurons *in vivo*. *Neurobiol Aging* **32**: e2311–e2315

- Kim J, Chu J, Shen X, Wang J, Orkin SH (2008) An extended transcriptional network for pluripotency of embryonic stem cells. *Cell* **132**: 1049–1061
- Lum L, Clevers H (2012) Cell biology. The unusual case of Porcupine. *Science* **337**: 922–923
- Malik B, Nie D (2012) Cancer stem cells and resistance to chemo and radio therapy. *Front Biosci (Elite Ed)* **4**: 2142–2149
- Verkaar F, Cadigan KM, van Amerongen R (2012) Celebrating 30 years of Wnt signaling. *Sci Signal* **5**: mr2
- Wend P, Fang L, Zhu Q, Schipper JH, Loddenkemper C, Kosel F, Brinkmann V, Eckert K, Hindersin S, Holland JD, Lehr S, Kahn M, Ziebold U, Birchmeier W (2013) Wnt/ β -catenin signalling induces MLL to create epigenetic changes in salivary gland tumours. *EMBO J* **32**: 1977–1989
- Yu Y, Ramena G, Elble RC (2012) The role of cancer stem cells in relapse of solid tumors. *Front Biosci (Elite Ed)* **4**: 1528–1541