Entity versus property: tracking the nature, genesis and role of stem cells in cancer

Conference on Stem cells and cancer

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The CNIO Cancer Conference on Stem cells and cancer took place between 23 and 25 February in Madrid, Spain, and was organized by E. Fuchs, M. Blasco, E. Batlle & M. Perez-Moreno.

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See Glossary for abbreviations used in this article.

Introduction

Decades after the elegant and groundbreaking experiments on the reversibility of the normal and tumorigenic phenotypes of germ cells and teratocarcinomas, and on vertebrate cloning and reprogramming by nuclear transfer (reviewed in Solter *et al*, 2006; Gurdon & Melton, 2008), the nature of stemness, cell plasticity and reprogramming is at

the forefront of biology. Stem-cell research promises to alter the face of medicine and our sense of self forever.

The CNIO Conference on Stem cells and cancer brought together an eclectic group of scientists to address the genesis, role and nature of stem cells in normal physiology, development and cancer. In her opening remarks, E. Fuchs (New York, NY, USA) suggested, for the purpose of the meeting, that the definition of a stem cell should include any cell that is able both to self-renew and to generate more differentiated cells. Over the subsequent three days, it became clear that no marker or set thereof can unequivocally identify a stem cell. In other words, stemness is a property rather than an entity. Here, we review a selection of presentations that—owing to space limitations—cannot be fully representative, but which nonetheless highlight some of the exciting topics presented at the meeting.

Asymmetric cell division and cancer

An important question is how stem cells regulate the balance between symmetric and asymmetric division, and between self-renewal and differentiation. Neural stem cells in *Drosophila* divide asymmetrically (Fig 1), segregating cell-fate determinants that block further stem-cell division and that instead induce differentiation. Mutations that disrupt this asymmetric segregation have been shown to cause tumours, as both daughter cells continue to proliferate at the expense of differentiation (Bello *et al*, 2006; Betschinger *et al*, 2006; Caussinus & Gonzalez, 2005; Choksi *et al*, 2006; Lee *et al*, 2006). The correct segregation of cell-fate determinants relies on cell polarity and on the alignment of these determinants over one spindle pole. The orientation of division is determined by centrosome positioning and, interestingly, abnormal centrosome function is often associated with human cancers (Fukasawa, 2007). C. Gonzalez (Barcelona, Spain) described the work of his group on the role of centrosomes in asymmetric division. His beautiful movies showed that one centrosome is fixed at the apical side of the cell while the basal centrosome wanders until it finds its appropriate position at the basal cortex, where the crescent of cell-fate determinants forms. His data strongly suggest that the MTOC might have a crucial role in the control of neuroblast polarity. Mutants that lack centrosomes cannot maintain their axis of division and, as a result, give rise to tumours (Castellanos *et al*, 2008). One

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of the cell-fate determinants that is segregated to the daughter cell is Brat, which is a Trim-NHL domain protein that acts as a tumour suppressor, inhibiting cell growth and proliferation. Brat has been shown to inhibit Myc in differentiating daughter cells, and mutations in Brat cause metastatic brain tumours (Betschinger *et al*, 2006). J. Knoblich (Vienna, Austria) has isolated the mouse homologue of Brat, TRIM32, and described that it also blocks cell proliferation and induces differentiation. TRIM32 interacts with Argonaute 1, which is a component of the RISC complex, and regulates the level of specific microRNAs such as LET7. TRIM32 also exhibits ubiquitin ligase activity, and targets Myc for ubiquitination and degradation. However, unlike mutations in Brat, the loss of TRIM32 does not cause tumours, perhaps owing to the continued presence of the related proteins TRIM2 and TRIM3 (Schwamborn *et al*, 2009).

Balancing symmetric and asymmetric division is crucial for the generation and maintenance of tissues, as well as to prevent tumorous overgrowth. A. Brand (Cambridge, UK) described the pattern of cell division in the optic lobe of the *Drosophila* larval brain, where symmetrically dividing neuroepithelial cells generate asymmetrically dividing neuroblasts (Egger *et al*, 2007). A comparison of the gene-expression profiles of these two types of neural precursor indicated that the Notch signal-transduction pathway is active in the neuroepithelium. When Notch signalling is blocked, cells drop out of the neuroepithelium and divide asymmetrically, suggesting that Notch maintains the neuroepithelium and prevents premature neurogenesis.

What regulates cancer stem-cell self-renewal?

The intercellular signalling pathways that are required for embryonic patterning are heavily involved in controlling stem-cell behaviour. Wnt signalling has been shown to be important in several organs. T. Piotrowski (Salt Lake City, Utah, USA) presented elegant work on the formation of the lateral line in zebrafish from a cluster of migrating stem cells and derived progenitors. During posterior migration, stem cells self-renew at the leading edge and give rise to clusters of cells that form sensory organs known as neuromasts, which are deposited periodically, thereby forming the lateral line. Neuromasts contain various cell types, including stem cells. A dynamic relationship between FGF and Wnt signalling ensures the correct temporal and spatial distribution of the cells that are involved in the partitioning of equivalent clusters to form neuromasts. However, how the stem cells themselves are regulated remains unclear. Fuchs also addressed the role of Wnt signalling, focusing on its function in the stem cells of the mouse hair-follicle bulge and its interactions with other pathways that maintain stem-cell quiescence. Her studies suggest that BMP signals contribute to maintaining the stem cells in a non-proliferative state. During the resting state of the hair follicle, Wnt/ β -catenin activity is dampened. Their activity coupled with a reduction in BMP signalling drives stem-cell activation. This suggests that a fine-tuned interplay might be responsible for supplying stem-cell progeny for homeostasis and repair, as well as for maintaining a pool of stem cells for as long as possible during life. It is widely suspected—although not yet proven—that ageing is greatly affected by the loss of stem-cell populations.

Wnt signalling also regulates stem cells in muscles and the gut. M. Rudnicki (Ottawa, Canada) presented work on the characterization of the nature and potential of muscle satellite stem cells in mice. Satellite cells are responsible for the postnatal growth and regeneration of skeletal muscle. He found that WNT7a is upregulated during

Fig 1 | Asymmetrical divisions in the *Drosophila* brain. The image shows a *Drosophila* larval brain lobe labelled with Discs large (red) to outline cells, BrdU (green) to mark dividing cells and DAPI (blue) to label DNA. The large red cells are asymmetrically dividing neural stem cells, which generate smaller, differentiating daughters that are shown in green and blue. Reproduced from Southall *et al* (2008). BrdU, bromodeoxyuridine; DAPI, 4',6-diamidino-2-phenylindole.

muscle regeneration and that exogenous WNT7a doubled the symmetrical division rate of satellite stem cells resulting in increased numbers of stem cells. The overexpression of WNT7a in muscle *in vivo* resulted in an astounding ~20% increase in muscle mass. These effects are specific for WNT7a—as WNT3a manipulation resulted in a smaller muscle mass—and seem to be mediated by the planar cell-polarity pathway rather than by the canonical or calcium-dependent WNT pathways (LeGrand *et al*, 2009). These important findings point to the control of cell polarity and the division plane as crucial determinants of stemness, and should pave the way for new and exciting studies. E. Battle (Barcelona, Spain) showed that the stem cells of the mouse intestine—which are found at the bottom of the crypt—have a stemness signature that is characterized by the expression of several WNT targets, including EPHB2 (Cortina *et al*, 2007). Such a signature might be useful to address issues of stem-cell biology in colorectal tumours.

There was a sense that general signalling themes in stem cells are becoming clear, with the HH and WNT pathways driving stemness, and BMPs, for example, acting as pro-differentiation antagonizing factors, although how these and other conserved signals act is context dependent. For example, BMP signalling is known to inhibit intestinal stem-cell self-renewal (He *et al*, 2004); however, J. Dick (Toronto, Canada) reported that, surprisingly, BMP4 induces human colon xenograft growth and Noggin inhibits growth, and that there is a good correlation between BMP4 levels and human tumour progression.

Glossary

BCR–ABL	breakpoint cluster region–Abelson leukaemia
BMI1	B-cell specific Moloney murine leukaemia virus-integration site 1
BMP	bone morphogenetic protein
Brat	brain tumour
CML	chronic myelogenous leukaemia
EPHB2	ephrin receptor B2
FGF	fibroblast growth factor
GBM	glioblastoma multiforme
Gli1	glioma-associated oncogene homologue 1
Hh	hedgehog
IFN α	interferon- α
IM	imatinib mesylate
INK4/ARF	inhibitor of kinase 4/alternative reading frame
iPS	induced pluripotent stem cell
K5	keratin 5
MEF	mouse embryonic fibroblast
MTOC	microtubule-organizing centre
NHL	protein domain with amino-acid sequence homology to Ncl-1, HT2A and Lin-41 proteins
NK	natural killer
OCT4	octamer 4; also known as POU class 5 homeobox 1
PTEN	phosphatase and tensin homologue
Rb	retinoblastoma
RISC	RNA-induced silencing complex
SCA1	stem-cell antigen 1
SOX2	SRY box 2
STAT1	signal transducer and activator of transcription 1
Terc	telomere RNA component
Trf2	telomere repeat factor 2
TRIM	tripartite motif
WNT	Wingless and Int

Much more research is needed to understand how normal and cancer stem cells control self-renewal, and how extracellular patterning information is interpreted by the responding stem cells in the appropriate niches. Interestingly, Polycomb-group genes have been found by several investigators to be involved in stem-cell self-renewal, probably acting downstream of extracellular patterning factors. For example, BMI1 can be regulated by HH signalling in some contexts (Leung *et al*, 2004), and M. Clarke (Stanford, CA, USA) presented data demonstrating a role of BMI1 in the self-renewal of long-term haematopoietic stem cells, as compared with short-term progenitors. BMI1 was also proposed to be crucial in prostate cancer. M. van Lohuizen (Amsterdam, the Netherlands) showed that transgenic mice expressing higher levels of BMI1 develop intraepithelial neoplasia in the prostate, similar to that of heterozygous PTEN mice (Trotman *et al*, 2003). However, BMI1 transgenic mice with a heterozygous deletion of PTEN develop full-blown prostate adenocarcinomas. This exciting finding might provide a reliable and relevant model for this type of cancer. In addition, van Lohuizen showed that HH signalling-induced medulloblastomas (Goodrich *et al*, 1997) do not develop in mice that lack BMI1, which show only early lesions that do not proceed to frank tumours.

Other genes that have been found to be crucial for stemness include members of the Sox family. Sox2 has been implicated in regulating the neural progenitor status during reprogramming, and Sox7 and Sox4 are Wnt targets expressed in cells that occupy the +4 position of intestinal crypts. Fuchs presented elegant work showing the essential role of Sox9 in hair-follicle bulge stem cells. By determining Sox9 expression, and using lineage tracing and pulse-chase

experiments, she could show that slow-cycling bulge stem cells form early in skin development, and that without Sox9 hair follicle and sebaceous gland morphogenesis are arrested, and wound repair is defective.

Tumour suppressors as stemness regulators

An exciting development was the convergence of views on the role of tumour suppressors in the regulation of stemness. M. Blasco (Madrid, Spain) reported that the epidermal stem-cell defects observed in mice with critically short telomeres (*Terc*^{−/−} third-generation mice or *K5–Trf2* mice) are rescued by the concomitant loss of p53 (Flores & Blasco, 2009; Stout & Blasco, 2009). How this occurs, however, is not known. A new and exciting twist—which builds on recent work by Zhao *et al* (2008)—was presented by K. Hochedlinger (Boston, MA, USA), who showed that the reprogramming efficiency of differentiated cells to iPS cells is greatly enhanced by the loss of p53. For example, *p53*^{−/−} melanocytes reached nearly complete reprogramming rates in secondary assays, similar to those obtained using immortalized melanocytes. Moreover, *p53*^{−/−} MEFs reprogrammed faster and at a higher frequency than wild-type cells. These iPS cells can form teratomas and chimaeras, showing a growth advantage with respect to wild-type cells.

A. Ruiz i Altaba (Geneva, Switzerland) presented a novel link between p53 and GLI1, the final mediator of HH signals that is also oncogene regulated and has a central role in human cancer (Stecca *et al*, 2007; Ruiz i Altaba *et al*, 2007). By using several model systems—including transgenic mice (Fig 2), mouse cortical and subventricular stem cells, epidermal hyperplasias and human gliomas—he described a general inhibitory loop between p53 and GLI1 that is involved in the control of stem-cell and tumour-cell numbers (Stecca & Ruiz i Altaba, 2009). Whether this pathway is involved in the regulation of skin stem cells or in the reprogramming described above remains to be tested.

In addition to p53, the other major tumour suppressor is the locus *Ink4/Arf*. This locus encodes p16, p15 and p19 (p14), which are important in controlling the p53 and Rb pathways. M. Serrano (Madrid, Spain) presented elegant data demonstrating that the *Ink4/Arf* locus not only acts as a tumour suppressor but also has a crucial role in the determination of lifespan. Counterintuitively, mice that moderately overexpress INK4/ARF show delayed ageing and increased longevity. The mechanisms that underlie the effects of INK4/ARF on stem-cell and organismal ageing are unresolved, although there is some evidence that cell-cycle inhibitors—such as p21—could be beneficial for the quiescence and long-term maintenance of adult stem-cell pools (Kippin *et al*, 2005).

Together with previous work on the role of p53 and PTEN in stem cells (for example, Groszer *et al*, 2001; Meletis *et al*, 2006), the new data indicate that the control of tumour development by tumour suppressors could be a result of their selected action as stemness regulators.

Is the number of cancer stem cells important?

As discussed above, stem cells could be defined by only two parameters: self-renewal, and the production of more differentiated progeny. In the light of this, it is interesting to consider whether the number of 'stem cells' is a relevant parameter, especially as it has been clearly shown that the niche or environment has a dominant effect. Indeed, A. Surani (Cambridge, UK) highlighted the existence of heterogeneous populations of embryonic stem cells that are in dynamic equilibrium

and respond to different environmental cues—such as culture conditions—as judged by the expression of *Stella*, which is a pluripotency and germ-cell marker (Hayashi *et al*, 2008).

Most grade III human gliomas—but not GBM—show a stemness signature that resembles that of embryonic stem cells—that is, high expression levels of *NANOG*, *SOX2* and *OCT4* (Clement *et al*, 2007)—which has subsequently been found in other tumours (Ben-Porath *et al*, 2008). Although the functional meaning of this signature remains to be addressed, Ruiz i Altaba reported that it is also observed in advanced colon cancers. If the strength of such a signature represents an increased number of cancer stem cells, grade III gliomas would contain a high abundance of cancer stem cells within the tumour population. By contrast, GBMs would have fewer stem cells in relation to the tumour mass and, therefore, their signature would be diluted, possibly because they not only self-renew, but also divide asymmetrically to self-renew and give rise to more differentiated progeny. This increase in total cell number could be the underlying factor for the fatality of GBMs.

Human metastatic melanomas have recently been shown to be highly enriched in cells that are able to initiate tumorigenesis in immunocompromised mice (Quintana *et al*, 2008), which was surprising in the light of previous data. S. Morrison (Michigan, MI, USA) reported that the difference—of several orders of magnitude—compared with previous work was due to his use of fully immunosuppressed mice lacking NK cells, which yielded frequencies of more than 20% of cancer-initiating cells. Dick similarly found that the deletion of NK cells increases leukaemic stem-cell engraftment frequencies. Interestingly, the use of these mice also enabled the isolation of additional populations fractionated on the basis of CD34 and C38 expression, which showed leukaemic stem-cell activity. These developments emphasize the need for *in vivo* functional assays to characterize cancer stem cells, and pave the way for more robust tests with rare and small tumour samples; Morrison even mentioned that his laboratory can now perform routine assays engrafting single cells.

But what does the frequency of cancer-initiating cells tell us about the original tumour? Probably not much more than a possible association with tumour grade, and malignant or recurrence potential, as the crucial—so far unaddressed—question about cancer stem-cell behaviour is one of fate, not numbers, in the original tumour. Nevertheless, the data presented clearly refute the idea that cancer stem cells need to be rare (Reya *et al*, 2001).

Cancer stem cells as targets of new therapies

Most types of advanced cancers remain incurable, either because they occur in vital organs—such as the pancreas or the brain—or because they have metastasized to distal organs and their eradication is ineffective (colon to liver, prostate to bone and so on). As cancer stem cells might be the cause of cancer recurrence and metastases, combinatorial therapies with agents that affect cancer stem-cell behaviour, and that kill these cells and their progeny, will probably have a major impact on patient care and real cure rates. For example, the synergistic effect of cyclopamine—a small plant alkaloid that selectively inhibits *Smoothened* and hence the *HH*–*GLI1* pathway that is involved in cancer stem-cell self-renewal—with the general DNA-alkylating chemotherapeutic agent temozolomide holds promise for the treatment of GBMs (Clement *et al*, 2007).

P. Dirks (Toronto, Canada) presented work aimed at standardizing and streamlining the testing of patient-derived GBM stem cells in

Fig 2 | Sagittal sections of the heads of embryonic day 14.5 wild-type (top panel) and bigenic Nestin-rtTA-IRES-LacZ; GFP-bitetO-GLI1 mice (bottom panel), stained with LacZ and eosin. LacZ labels the normal progenitors in the wild-type mouse, and the expanded population of progenitors and stem cells in the mouse, which express higher levels of GLI1. Reproduced from Stecca & Ruiz i Altaba (2009). bitetO, bidirectional tetracycline operator; Cb, cerebellum; Ctx, cortex; GFP, green fluorescent protein; GLI1, glioma-associated oncogene homologue 1; IRES, internal ribosome entry site; LacZ, β -galactosidase; Md, medulla; rtTA, reverse tetracycline-controlled transactivator; Sc, spinal cord; Tct, tectum; Thal, thalamus.

real time, partly through the efficient generation of adherent cultures on laminin, which increases the purity of cancer stem-cell isolation. Such methods should allow the isolation of compounds that inhibit GBM stem-cell self-renewal for their use in the clinic (Pollard *et al*, 2009). These approaches are crucial, as cancer treatments that are based exclusively on classical chemotherapeutic agents are not sufficient. To make matters worse, Dick reported that oxaliplatin treatment of colon cancers actually boosted the abundance of cancer stem cells by more than 10 times.

In this sense, A. Trumpp (Heidelberg, Germany) addressed the issue of stem-cell dormancy, which might offer a new treatment strategy for leukaemias such as CML (Wilson *et al*, 2008; Essers *et al*, 2009). He characterized the previously unrecognized population of dormant 'mother-of-all' long-term hematopoietic stem cells in mice, which under homeostatic conditions divide only around five times during a lifetime. This behaviour is in contrast to activated stem cells,

which show slightly reduced self-renewal activity and divide approximately once per month. Importantly, Trumpp showed that IFN α –STAT1–SCA1 signalling activates dormant stem cells making them sensitive to antiproliferative chemotherapy. Interestingly, before the approval of the BCR–ABL inhibitor IM in 2001, CML patients were treated with a long-term high dose of IFN α , which was often ineffective although some cures were reported. IFN α has been replaced in recent years by IM, which unfortunately has to be provided for life, as its discontinuation results in a high relapse rate, apparently because dormant CML stem cells are resistant to IM. Trumpp suggested that CML patients should initially be treated with a short pulse of IFN α to activate and sensitize dormant CML stem cells before IM therapy is initiated. Such a strategy could be effective, as suggested by the results of a French trial in which six patients who were initially treated with IFN α were subsequently switched to IM and showed a surprisingly high rate of long-term complete remission even after stopping IM therapy (Rousselot *et al*, 2007).

In summary, the CNIO Stem cells and cancer meeting yielded an emergent synthesis of the multiple and combined efforts to understand stem-cell behaviour in cancer. Crucially, it also highlighted how excellent stem-cell science guides new anticancer efforts.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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