

Abstracts

Role of mathematical modeling in deciphering crucial fate decision proteins in cancer stem cells and in harnessing them to therapy

Zvia Agur, Oleg Kirnasovsky, Yuri Kogan, Liora Levi, Lilach Tencer-Hershkovitz, Genadiy Vasserman

Institute for Medical Biomathematics (IMBM), Bene Ataroth, Israel

Mathematical theory, predicting that homeostasis is achieved by a negative feedback on stem cell (SC) proliferation, implies a Quorum Sensing mechanism in higher vertebrates [1]. A Quorum Sensing mechanism was implemented in a Cellular Automata model of tissue development and computer simulations were performed for each set of tissue parameters. Results show a sharply dichotomous growth dynamics: maturation within 50 - 400 cell-cycles or immortalization. This dichotomy is driven by a hierarchy of factors, primarily intercellular communication: low inter-stem cell communication leads to perpetuation of the proliferative state. These results suggest that cancer SC (CSC) proliferation can be attenuated through signal manipulation, or enhanced through cytotoxics targeted to differentiated cells [2].

In the next stage, we studied the complex intracellular interactions governing normal and pathological mammary SC fate decision. Our aim was to identify the crucial factor(s), whose modulation can redirect CSCs into final differentiation. Our mathematical model is a system of 7 differential equations describing the major signaling pathways governing SC development and their interactions. Using this model we numerically simulated the effect of in situ Dkk application on normal SCs proliferation and on that of mammary CSCs. Our simulation results suggest a biphasic effect for Dkk on SCs: Low Dkk levels accelerate SC proliferation, whereas high Dkk levels drive SCs into differentiation, ultimately leading to tumor elimination. Our findings suggest that treatment with Dkk can promote CSC differentiation and drive the cancerous tissue to exhaustion. Validation of our suggestion in preclinical and clinical research will clarify the efficacy of this new therapy strategy. Moreover, our results imply that breast cancer metastasizing cells, which bear mutations in the Wnt pathway, and hence over-express Dkk, can annihilate bone osteoblasts while preserving their own augmented proliferation [3, 4]. Laboratory experiments in mammospheres corroborate the theoretical results [Clarke et al., in preparation].

1. Agur Z., Daniel Y., Ginosar Y. The universal properties of stem cells as pinpointed by a simple discrete model. *Jour. Math. Biol.*, 44 (1), 2002 (pp.79-86).
2. Agur, Kirnasovsky, Levy (submitted) Normal and pathological renewal of damaged tissues by stem cell homing: a simulation model.
3. Kirnasovsky O.U., Kogan Y, Agur Z. Analysis of a mathematical model for the molecular mechanism of mammary stem cell fate decision. *Mathematical Modelling of Natural Phenomena* (in press).
4. Oleg U. Kirnasovsky, Lilach Tencer, Genadye Vasserman, Zvia Agur. Dickkopf (Dkk) protein as a candidate for diverting proliferating cancer stem cells to terminal differentiation: Evaluation by a mathematical model. *American Association for Cancer Research, AACR, Mar 16-19, 2008 Dead Sea, Jordan*, pp 28

Novel Wnt/beta-catenin target genes in epithelial plasticity and invasive tumor development

Avri Ben-Ze'ev

Department of Molecular Cell Biology, Weizmann Institute of Science, Rehovot, 76100, Israel

Recent studies have pointed to a direct link between epithelial mesenchymal transition (EMT), the gain of cancer stem cell properties and the escape from failsafe programs during the acquisition of invasive features by epithelial cancer cells. We have addressed the plasticity in the phenotype of invasive tumor cells in colorectal cancer (CRC) by investigating the dual role of beta-catenin in the cell. Aberrant beta-catenin signaling is prevalent in most CRC patients. Beta-catenin, an important molecule in cell-cell adhesion, binding cadherin receptors to the cytoskeleton, is also a key co-transcriptional activator of target genes (in complex with TCF factors) in the nucleus. Hyperactive beta-catenin induces genes regulating the cell cycle and the invasion and metastasis of cancer cells. We studied the coordination between cell-cell adhesion and signaling by the E-cadherin/beta-catenin complex during invasive CRC development. We found that sparse cell cultures mimic cells at the invasive front of tumors displaying low levels of E-cadherin, but highly active nuclear beta-catenin that transactivates Slug, a negative transcriptional regulator of E-cadherin. Slug is also induced during EMT in embryonic development. This cell density-regulated change in beta-catenin and E-cadherin is similar to that observed at the invasive versus differentiated areas of human CRC tissue. We identified Nr-CAM and L1, two members of the IgCAM receptor family (normally expressed in nerve cells), as novel target genes of beta-catenin in CRC, and found them exclusively localized at the invasive front of CRC tissue. Forced expression of L1 and Nr-CAM in fibroblasts induces motility, cell transformation, and confers tumorigenesis in mice and, in CRC cells, causes liver metastasis. Suppression of L1 and Nr-CAM in CRC cells reduces cell motility and invasion. Expression of the Nr-CAM ectodomain was sufficient for cell growth in low serum, causing constitutive activation of the MAPK/ERK and AKT pathways and further, could transform NIH3T3 cells that formed tumors in mice. We detected both L1 and the metalloproteinase ADAM10 (a novel beta-catenin target gene) that cleaves the L1 ectodomain, at the invasive front of CRC tissue, and showed that their co-expression in CRC cells enhances metastasis to the liver. We suggest that CRC cells exploit opportunistically these “neuronal” cell adhesion molecules, whose transcription is aberrantly activated by beta-catenin - to promote metastasis. Since the ectodomains of L1 and Nr-CAM are often shed by metalloproteinases, they could serve as diagnostic markers and anticancer therapy targets.

Growth of MCF-7 cells in 1, 2 and 3 dimensions.

Björn Boysen

Fraunhofer Institute for Biomedical Engineering, Potsdam-Golm, Germany

In order to supply experimental data for the the computer simulation of proliferation kinetics, the growth of MCF-7 breast cancer cells was observed over several days using time-lapse videography in high resolution. Image sets were analysed to yield 1D, 2D and 3D growth curves and then passed on to the IMBM group for further processing.

Experimental approaches to quorum sensing of MCF-7 mammospheres

Björn Boysen

Fraunhofer Institute for Biomedical Engineering, Potsdam-Golm, Germany

It is assumed that an MCF-7 population is not homogeneous but comprises at least cancer stem cells (CSC) and differentiated tumour cells (DC). The hypothesis of quorum sensing acts on the assumption that every cell perceives and is influenced by its direct neighbourhood. For example, a reduction of DC could be perceived by the CSC and activate differentiation until the original state is reached. Vice versa, a reduction of CSC could induce symmetric division of the remaining CSC to recover the losses. To investigate such relations, CSC and DC would ideally be live stained, selectively eliminated and then observed. However, it turned out to be experimentally difficult to distinguish living CSC and DC in one culture. Therefore, we sorted MCF-7 cell populations for CSC-associated markers and compared CSC-enriched with CSC-depleted cultures for their tumour formation capability which was deduced from mammosphere-formation assays. The used markers were a) expression of CD44 which cells were sorted for by MACS and b) cell size, which cells were separated for using an elutriator.

Regulation of breast cancer stem cells: the potential for novel therapies

*Robert Clarke, Sacha Howell, Ciara O’Brien, Rebecca Lamb, Kath Spence, Hannah Harrison
and Gillian Farnie*

Breast Biology Group, School of Cancer and Imaging Sciences, Paterson Institute for Cancer
Research, University of Manchester, Wilmslow Road, Manchester, M20 4BX, UK.

We have been investigating human breast cancers for the presence of a stem-like cell population. Using both adherent and non-adherent culture methods known to enrich for normal tissue stem cells, we have demonstrated that breast cancer cell lines and primary tumours contain a self-renewing, colony-forming population that can be enriched for by cell surface markers such as CD44, alone or in combination with other markers. We have demonstrated this tumorigenic stem-like cell population to be regulated by the epidermal growth factor (EGF) and Notch receptor signaling pathways.

In order to discover novel pathways involved in the control of breast cancer stem-like cells (CSCs), we have applied retroviral short hairpin (sh) RNA libraries in a genome-wide loss of function screen along with gene expression arrays. This integrative genomic analysis has revealed the importance of the Wnt and PI3K/Akt signalling pathways as well as several others not previously known to regulate stem cell survival and self-renewal.

There is good evidence that CSCs in breast and other tissues are resistant to radio- and chemotherapy suggesting that CSC-specific treatments are needed. We propose that differentiating agents, inhibitors of CSCs or their resistance pathways could represent a new therapeutic modality in breast cancer, perhaps in combination with current treatments.

Approaches for controlling the microenvironment of cells

Claus Duschl

Fraunhofer Institute for Biomedical Engineering, Potsdam-Golm, Germany

One of the big challenges in modern cellular biology is to understand and control the mechanisms that determine cell fate and cell behaviour. In particular, a better control over differentiation processes would open up new possibilities for addressing important issues in biomedicine and pharmacological research. Obstacles are our incomplete understanding of intracellular signalling and the lack of experimental approaches that allow a sufficient control over the microenvironment of individual cells. This microenvironment constitutes the entire pool of information that is available to the cell. At the Fraunhofer Institute for Biomedical Engineering, we are aiming at developing devices for the handling and manipulation of cells where their microenvironment is tightly controlled. This can be achieved by employing microfluidic channels that allow the generation of concentration patterns of soluble signalling molecules and by carefully engineering surfaces the cell can have physical contact with during manipulation. Another important approach concerns the precise geometrical arrangement of individual cells within a cluster. It allows the investigation of intercellular exchange of information through soluble signal molecules such as paracrine factors and may provide a promising tool to predict the development of cell clusters such as solid tumours. In this contribution, we present a couple of approaches towards these goals ranging from simple lab protocols to high-tech assemblies. Assumptions for in-silico modelling were substantiated by e.g. time lapse measurement of cell growth kinetics, analysis of tumoursphere generation and experimental support for the "quorum sensing" theory.

Therapy resistance in cancer stem cells: is differentiation therapy the answer?

Gillian Farnie, Hannah Harrison and Robert Clarke

Breast Biology Group, School of Cancer and Imaging Sciences, Paterson Institute for Cancer Research, University of Manchester, Wilmslow Road, Withington, MANCHESTER, M20 4BX, United Kingdom

Recent reports have shown that cancer stem-like cells (CSCs) from solid cancers are resistant to both radio (RT) and chemotherapy (CT). CSC enrichment has also been observed in residual or recurrent cancers after treatment with RT or CT.

MCF7 cells contain a cell population with cell surface markers $ESA^+/CD44^+/CD24^{low/-}$, which are enriched for cells that can initiate mammospheres (MS). This suggests a high proportion of mammosphere initiating cells (MSIC) have the cells surface markers $ESA^+/CD44^+/CD24^{low/-}$. Using a clonogenic survival assay, and mammosphere culture, we observed MCF7 cells enriched for MSIC are significantly more radio-resistant than cells grown in the same conditions from monolayer. Similar results were observed in primary breast cancer cells (n = 4, 2 DCIS and 2 pleural effusion), where MSIC from the dissociated primary tissue had a significant increase in MS survival after IR compared to the freshly digested cells.

The $CD44^+/CD24^{low/-}$ cell population of the MCF7 cell line increased after fractionated IR. This *in vitro* data mimics recent studies *in vivo* where treatment with RT or CT has caused an increase in the CSC population in a number of different cancers. Differentiation of the population of CSC might therefore increase sensitive to current treatment regimes of RT and CT, potentially eliminating a future recurrence. Using MCF7 and a primary sample we show that after treatment the differentiating agents, sodium butyrate or retinoic acid, the $CD44^+/CD24^{low/-}$ population can be reduced with a corresponding increase in the more differentiated $CD24^+$ cell population. MCF7

cells pre-treated with sodium butyrate or retinoic acid were also found to be more sensitive to IR when grown in MS culture.

These data indicate that MSIC are more radio-resistant than the whole cell population and that differentiation agents can reduce the MSIC enriched population. This suggests that adjuvant differentiation therapy combined with RT or CT may help reduce recurrence of tumours.

Mathematical Model for the Molecular Mechanism of Fate Decision in Neural Stem Cells

Yuri Kogan, Karin Halevi, Zvia Agur

Institute for Medical Biomathematics (IMBM), Bene Ataroth, Israel

Most malignant brain tumors have poor prognosis even when treated with aggressive therapy. Recently, stem-like cancer cells with unlimited self-renewal capacity and the ability to regenerate a tumor were isolated from brain tumors. These brain tumor stem cells (BTSCs) were found to be responsible for the tumor re-grow, metastasis and clinical relapse. Therefore, BTSCs could become ideal targets for brain tumor treatment. One approach would be to force the differentiation of BTSCs, by depleting this CSC population, decreasing tumor initiating and self-renewal capacity.

Molecular analysis of the BTSCs may lead to the identification of pathways important for proliferation, differentiation and self-renewal of these cells and recognize new targets for therapy. Some of the major neural developmental pathways (WNT, Notch, TGF- β , BMP and Hedgehog) have been implicated in brain tumors and have the potential to be involved in BTSC-mediated tumor initiation and progression. BMP may cause differentiation in some tumors and/or cell lines while it can increase proliferation of others. Thus it may be difficult to predict the effect of BMP signaling pathway on proliferation or differentiation of BTSC.

We have developed a model for the main signaling pathways (Wnt, Notch and Shh) regulate BTSC proliferation and differentiation and the interactions between them. We used mathematical modeling for understanding the complex and interconnected intracellular pathways responsible for the fate decision of neural stem cells. As part of the parameter estimation for this mathematical model we developed a biochemical and mathematical model for the first steps of the Wnt signaling pathway: binding of Wnt to its receptors, the effect of SFRP and DKK on Wnt binding and β -catenin accumulation.

Investigation of Quorum Sensing of Breast Cancer Stem-like Cells using the MCF7 cell line

Rebecca Lamb, Hannah Harrison and Robert Clarke

Breast Biology Group, School of Cancer and Imaging Sciences, Paterson Institute for Cancer Research, University of Manchester, Wilmslow Road, Withington, MANCHESTER, M20 4BX, United Kingdom

Quorum sensing describes the phenomenon where a cell senses its environment through the concentration of signalling molecules indicating the number of surrounding cells. Based on the information that a cell receives, a decision will be made whether it is to self-renew or differentiate. The hypothesis is that if the number of stem-like cells within a population is decreased, the stem cells will equilibrate over time and achieve steady state levels through quorum sensing.

To test this, the MCF7 breast cancer cell line is being used as a model for breast cancer and stem-like cells. Cells are sorted by flow cytometry based on the cell surface expression of CD44, CD24 and

ESA. CD44⁺/241o/ESA⁺ stem-like cells are separated from the differentiated cell population. These have been shown to represent breast cancer stem-like cells, which produce increased numbers of mammospheres (MS). After sorting, different proportions of the sorted cell populations are plated in adherent culture and incubated at 37^o C for up to one week. At various time points, we are analysing CD44⁺/241o expression and MS formation in order to assess stem cell activity. Results using this model system will be presented.

In conclusion, MCF7 can be used as a useful tool to assess cell population growth and quorum sensing of “breast stem-like cells”.

Wnt signalling pathway and its role in the regulation of breast cancer stem-like cells

Rebecca Lamb, Robert Clarke

Breast Biology Group, School of Cancer and Imaging Sciences, Paterson Institute for Cancer Research, University of Manchester, Wilmslow Road, Withington, MANCHESTER, M20 4BX, United Kingdom

Evidence suggests that breast cancers contain tumourigenic stem cells or stem-like cells and that de-regulation of their self-renewal plays a role in disease development.

Growing data suggests a role of the Wnt pathway through upregulation in breast cancer. The aim was to explore this further and investigate the role of Wnt in breast cancer stem-like cells.

Using qRT-PCR, I extensively investigated the mRNA expression of 26 genes within the Wnt pathway using 6 normal, 6 ER⁺ve, and 5 ER⁻ve breast cancer cell lines and identified statistically significant differences ($p \leq 0.05$). Upregulation of Wnt targets Axin2 and Lef1 were observed in breast cancer cell lines and a decrease in the inhibitor DKK1. Significant differences were observed between ER⁺ve and ER⁻ve cell lines with a decrease in Wnt5a, Wnt5b, WISP1, LBH and sFRP1 in ER⁺ve.

Cells from the breast cancer cell line MCF7 were cultured using an *in vivo* non-adherent system to grow mammospheres (MS) which enriches for self-renewing stem-like cells. Cells were harvested after 1/4/7 days and Wnt pathway mRNA expression compared to adherent conditions. MSs showed a clear increased expression of the Wnt pathway.

MCF7s treated in monolayer with DKK1 (1ng/ml -100ng/ml) for 48/96 hrs showed a significant reduction in MS formation ($p \leq 0.05$).

The Wnt pathway plays an important role in breast cancer with differential expression dependant on ER status and is involved in the self-renewal of breast cancer stem-like cells. Finally, inhibition using DKK1 significantly decreases the self-renewal of the stem-like cells.

A “Devo-Patho” approach to stem cell differentiation: From embryonic stem cells to brain cancer stem cells.

Luc Leyns

Lab of Cell Genetics, Vrije Universiteit Brussel, Brussel, Belgium

Embryonic stem (ES) cells are pluripotent and have the capacity to differentiate into every cell type of the adult body. Therefore, they have great potential in regenerative medicine, but also they provide an excellent tool to study embryonic development. The challenge is to alter differentiation toward functional cell types or tissues by directing ES cells to a specific fate. Efforts have been made to understand the molecular mechanisms that are required for the formation of the different germ layers and tissues from ES cells, and these mechanisms appear to be very similar in the mouse embryo.

We have been studying the differentiation toward mesoderm and mesoderm derivatives by analyzing the roles of Activin A/Nodal, BMP, and FGF signaling using a dual ES cell differentiation system combining a loss-of-function with a gain-of-function approach. We found that Activin A or Nodal directed the nascent mesoderm toward axial mesoderm and mesendoderm, while Bmp4 was inducing posterior and extra embryonic mesoderm at the expense of anterior primitive streak cells. FGF signaling appeared to have an important role in mesoderm differentiation by allowing an epithelial-to-mesenchymal transition of the newly formed mesoderm cells that would lead to their further patterning.

Another question we tackled is to understand how neural induction occurs and how the neural tissue is patterned along the antero-posterior (A/P) body axis. In this work we designed a gain-of-function/loss-of-function approach using mouse ES cells to study this early neural patterning. Our data confirm that in the mouse, two steps are involved in neural patterning, induction of anterior neural tissue and posteriorization and we show that while Fgf4, Fgf8 and Wnt1 have no strong patterning effect, Fgf2, Wnt3a and Bmp4 are strong posteriorizing factors.

Over the past few years, evidence has accumulated supporting the hypothesis that cancer robustness may be attributed to a small portion of the tumor mass, the cancer stem cells (CSCs) suggesting that cancer may be a stem cell disorder. The proliferative, self-renewal and differentiation properties of these CSCs are reminiscent of normal stem cells, suggesting that they may behave similarly when placed in presence of the same signals. This leads us to propose a more target-oriented treatment for cancer based using embryonic stem cells as a paradigm.

Our approach is geared towards the addition of signaling factors in the microenvironment of the CSCs that will lead to their differentiation into non-proliferative and non-malignant cells. We focus in particular on cancer from neural origin such as glioblastoma. While brain tumors only account for about 2% of human cancers, it is responsible for a large number of deaths. In particular, glioblastoma is the most aggressive form of brain cancer characterized by a high vascularisation and infiltrating capacity. It has a poor prognosis in patients and the median survival of the patients after treatment (radio- and chemotherapy) is only 14,6 months. We are presently testing the effect on glioblastoma CSCs of a series of secreted factors that activate specific signaling pathways as well as secreted antagonists of pharmacological inhibitors of these pathways.

This “Devo-Patho” approach consists of applying the knowledge gained by studying developmental processes such as the proliferation, maintenance and differentiation of embryonic stem cells to pathological situation such as brain cancer and in particular the cancer stem cells which have been proposed to be a key component in a series of tumors.

Controlling the local environment of differentiating stem cells

Christine Mibler

Fraunhofer Institute for Biomedical Engineering, Potsdam-Golm, Germany

Since cell-cell communication occurs via paracrine factor secretion and reception, it was questionable whether stem cell differentiation would be possible inside a microchannel. Under constant flow conditions, putative paracrine factors can be removed, thus preventing signalling events which are crucial for differentiation. To challenge this hypothesis, we set up a microfluidic system for the differentiation of mouse embryonic stem cells.

For our experiments, we induced E14Tg2A cells to differentiate into neural and cardiac direction. Differentiation was assessed by morphology and immunofluorescence detection of specific marker proteins.

It is assumed that differentiating cells perceive their neighbours' presence via reception of secreted paracrine factors. To test this hypothesis, we altered the effectiveness of paracrine signalling by physically limiting molecular diffusion in the culture medium. By increasing medium viscosity or by overlaying the cell lawn with solid agarose, a significant increase of stem cell differentiation efficiency could be achieved as it was confirmed by morphological assessment and Western Blot analysis. This suggests an important role of diffusing factors for cell-cell communication for differentiation and is well consistent with the idea of quorum sensing. Serendipitously, our agarose coating method is a practical improvement to hitherto employed protocols.

Cells in Gels: Exploring Cellular Morphogenesis and Differentiation in 3-D Culture

Dror Seliktar

Faculty of Biomedical Engineering, Technion – Israel Institute of Technology

The regulation of cellular morphogenesis and differentiation via the physical properties of the provisional extracellular matrix (ECM) is poorly understood and our group has been working towards elucidating the dominant physical factors of the ECM that influence cell spreading, migration and differentiation in 3-D culture. We apply a biosynthetic PEG-protein hydrogel as an ECM-analog for cell culture, with highly defined and precisely controllable density, microarchitecture, proteolytic susceptibility, compliance and biofunctionality. The matrix is used to encapsulate mesenchymal cells while pseudo-independently altering biochemical and physical properties of the microenvironment using simple compositional modifications to the bio-synthetic constituents. We have shown that the proteolytic resistance and compliance of the matrix have a profound influence on the regulation of cell morphogenesis and phenotype determination. Beyond the control over the intrinsic physical attributes of the hydrogel, our laboratory has recently developed an optical 3-D micro-patterning approach to non-invasively create any prescribed geometrical feature having submicron spatial resolution in situ, anywhere within the PEG-protein hydrogel biomaterial. The micropatterns are made using a simple but highly effective application of computer-guided laser micro-ablation that creates localized imperfections in the hydrogel architecture. These imperfections are used to guide anisotropic cellular development within the amorphous material, including preferentially guiding neural cellular development in the hydrogels based on contact guidance and differential mechanical resistance of the scaffolding. Precisely controlled bulk material properties and custom 3-D landscaping with micropatterning are collectively used to elucidate the dominant and influential physical factors affecting morphogenesis patterns, phenotypic states, and differentiation of various cell types.

General law of tumor growth and efficacy of cancer therapy: mathematical modeling and analysis of experimental data

Oleg Kirnasovsky, Vladimir Vainstein, Yuri Kogan, Zvia Agur

Institute for Medical Biomathematics (IMBM), Bene Ataroth, Israel

Cancer stem cell (CSC) hypothesis states that only a small fraction of a malignant cell population is responsible for tumor growth and relapse. Understanding the interactions between stem cells (SCs) and non-SCs may contribute to improving success in cancer elimination.

Our aim in this work is to analyze the dynamics of the established cancer population and to examine new therapeutic avenues for eliminating CSC. We increased the realism of the previous discrete model by developing a generalized probabilistic Cellular Automata (CA) model. The model treats two populations of cells: SCs that can divide indefinitely, and differentiated cells (DCs) that do not

divide and have a limited lifespan. We assume negative feedback on CSCs proliferation by total cell density, and activation of CSCs differentiation by high CSCs density. We simulated the model under wide range of physiologically feasible initial conditions and parameter values.

The simulations showed that growth of cancer cell colonies should follow power law dynamics rather than exponential or Gompertzian growth, due to constant rate of colony radius increase. In vitro experiments with MCF-7 cell line showed that the growth of cell colonies both in one and two dimensions displays linear radius growth, as predicted by the model. Moreover, analysis of growth of EMT6 breast cancer xenografts in mice led to the same conclusion.

We analyzed efficacy of different regimens of cancer radiotherapy in cases of exponential versus power law tumor growth. We conclude that specific type of general tumor growth law can influence the choice of radiotherapy schedule.

The stem state in cancer: tumor-initiating cells are biologically distinct from stem cells

Dov Zipori

Department of Molecular Cell Biology, Weizmann Institute of Science, Rehovot 76100, Israel

Tumors contain cells that are capable of extensive proliferation in vitro, and may also form tumors in animal models upon in vivo inoculation. These cells have been designed as the tumor initiating cells (TIC). Recent studies suggested the “cancer stem cell” theory, namely, that TIC are in fact stem cells. This is deduced from the rareness of these cells among the tumor population, molecular markers shared with normal stem cells and the high proliferation capacity that TIC exhibit. However, this assumption suffers from some major inconsistencies. TIC lack the delineating traits of stem cells; TIC exhibit reduced or absent differentiation potential. Any attempt to define normal stem cells without reference to their wide range differentiation potential will leave the stem cell definition hollow since pluripotent differentiation is a hallmark of stemness. Normal stem cells reside in specific niches, which antagonize their differentiation and allow their self-renewal. The stem cell niche keeps normal stem cells quiescent and stem cell divisions are a very rare occurrence. This major stem cell trait is absent in TIC that proliferate continuously and in an autonomous manner. Normal stem cells and TIC represent therefore two extremes in biological terms. The differences between cells in the stem state, and TIC, represent the divergence between normal tissues and tumors.