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Towards a quantitative understanding of stem cell–niche interaction: Experiments, models, and technologies

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ABSTRACT

Here we report about an interdisciplinary workshop focusing on the effects of the local growth-environment on the regulation of stem cell development. Under the title "Towards a quantitative understanding of stem cell/ niche interaction: Experiments, models, and technologies", 33 experts from eight countries discussed current knowledge, new experimental and theoretical results as well as innovative measurement technologies. Specifically, the workshop addressed the following questions: What defines a stem cell niche? What are functional/regulatory characteristics of stem cell– microenvironment interactions? What experimental systems and technologies for quantifying niche function are available?
As a consensus result it was recorded that there is no unique niche architecture across tissues but that there are generic principles of niche organization guaranteeing a proper function of stem cells. This functional aspect, as the major defining criterion, leads to the conclusion that stem cells and their niches need to be considered as an inseparable pair with implications for their experimental assessment: To be able to study any of those two components, the other component has to be accounted for. In this context, a number of classical in vitro assays using co-cultures of stem and stroma cells, but also new, specifically bioengineered culture systems have been discussed with respect to their advantages and disadvantages. Finally, there was a general agreement that the comprehensive understanding of niche-mediated stem cell regulation will, due to the complexity of involved mechanisms, require an interdisciplinary, systems biological approach. In addition to cell and molecular biology, biochemistry, biophysics and bioengineering also bioinformatics and mathematical modeling will play a major role in the future of this field.

Introduction

"The stem cell niche is a phrase loosely used in the scientific community to describe the microenvironment in which stem cells are found, which interacts with stem cells to regulate stem cell fate." [1] This statement from the Wikipedia Website on the "stem cell niche" (May 2010) adequately pinpoints the current standard of knowledge in stem cell biology, if the discussion comes to the stem cell niche. Although microenvironmental cues are generally accepted as major factors regulating stem cell function, and although many particular regulatory mechanisms and pathways of cell/microenvironment interaction are known, a global picture of stem cell–microenvironment interaction and its impact on stem cell fate regulation is still missing. Thus, there is an obvious need for a systematic analysis of known facts, of open questions, and of available techniques and technologies to approach these questions.

Here we report on the discussions and the results of an International Workshop on Concepts and Models of Stem Cell Organization that was held from May 17th to 18th, 2011 in Dresden, Germany, which particularly aimed at such a systematic survey of the

Abbreviations: BM, bone marrow; CAFC, cobblestone area-forming cells; ECM, extracellular matrix; EGF, epidermal growth factor; HSC, hematopoietic stem cells; LTC, long-term culture; MSC, mesenchymal stem cells; PCA, principal component analysis; TCR, T-cell receptor; TNF, tumour necrosis factor; TPO, thrombopoietin.

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current state-of-the-art in the field of stem cell–niche interaction. The meeting was the 5th in a series of conceptual workshops, termed *StemCellMathLab*,[2] which have the objective to provide an interdisciplinary platform for scientists of diverse backgrounds to discuss specific topics in the area of stem cell biology. The *StemCellMathLab* 2010, which was jointly organized by the Institute for Medical Informatics and Biometry, Medical Faculty Carl Gustav Carus, Dresden University of Technology and the Institute for Medical Informatics, Statistics and Epidemiology, Medical Faculty, University of Leipzig, brought together 33 scientists from eight countries, with scientific backgrounds comprising medicine, cell biology, bioengineering, chemistry, bioinformatics and mathematics. In particular, the workshop addressed the following topics: (1) What defines a stem cell niche? (2) What are functional/regulatory characteristics of stem cell/microenvironment interactions? (3) What experimental systems to study stem cell/ niche interactions are available? (4) What techniques and technologies for quantifying niche function are available?

In this publication we report on the major discussions of the workshop. While the section "Defining the stem cell niche" summarizes characterizing criteria of a stem cell niche and describes different niche concepts, section "Quantifying the niche" provides an overview of currently available methodologies to access stem cell/niche interactions. Beyond different experimental techniques the section "Modeling the niche" highlights a number of theoretical and computational approaches. The report is closed by a summary section.

Defining the stem cell *niche*

It is widely accepted that there is a concerted interplay of tissue stem cells and their local microenvironment which is necessary for the correct regulation of homeostatic maintenance as well as for tissue regeneration after disturbance or injury [2,3]. Although this general principle seems to be valid for most (if not all) regenerative tissue types, it has to be expected that the specific realization of this principle is rather diverse. Such diversity is, above all, suggested by the obvious differences in the anatomical regions in which different tissue-specific stem cells are found. Given the extremely heterogeneous architecture of potential niche structures, the question arises whether there is something like a common, tissue-independent niche characterization. Answering this question is important, as it will determine whether a unique niche definition can be expected or whether a multitude of different niche definitions (e.g. each for every tissue type) is necessary? This question also leads directly to the problem, whether the niche needs to be perceived as a real entity or whether it should be interpreted as a (theoretical) concept. Similarly to the question above, the answer obviously affects the nature of the niche definition: either referring to specific (e.g. molecular or physical) components or focusing primarily on general features.

Tissue specificity of the stem cell niches

In this section we summarize known facts and available data on stem cell niche concepts for a number of important tissue types. The selection of tissues is not complete; it represents the topics discussed during the workshop.

Bone marrow (hematopoietic stem cells)

Hematopoietic stem cells (HSC), which can be found in the bone marrow (BM), represent one of the best-studied stem cell populations. The existence of a particular BM niche has been suggested as there is evidence for the existence of characteristic regulatory regions within the BM mediating regulatory signals from stromal cells and extracellular matrix (ECM) components [2–4].This perception of an inductive HSC microenvironment was already introduced in the early 1970s by John Trentin [5,6] and Raymond Schofield [7], the latter introducing the term niche for the first time in 1978. Following this interpretation, a niche corresponds to a defined anatomical region regulating stem cell function by means of secreted and cell surface molecules, mechanical signals, spatial arrangements, and particular metabolic conditions [8,9]. For the bone marrow, two distinct HSC-supporting regions have been proposed recently: the osteoblastic niche [4,10] and the vascular niche [11]. However, the specific functions of these anatomical sites remain unclear, and it is still under debate whether HSC are able to reversibly change between those niche environments, and how such HSC regulation is mediated in this particular context. There are a number of known molecular pathways that are involved in the cross-talk between HSC and their microenvironment (e.g. Wnt-pathway, Notch, Ang1/Tie2 interaction, or SCF/c-Kit), but only incomplete knowledge has been accumulated on how those pathways effect HSC fate. Another open question in the hematopoietic system is the effect of the spatial arrangement of HSC and niche components, such as osteoblasts, vasculature, and ECM components. In contrast to other tissues, with an obvious anatomically defined stem cell microenvironment, the HSC niches are still only vaguely defined. However, our knowledge is currently starting to increase considerably, e.g. by the availability of in vivo imaging technologies allowing to monitor HSC behavior within its natural environment [12].

Blood/lymph (T-cells)

In contrast to other blood cell lineages, T-cells are able to maintain their population and to produce different T-cell subtypes without a considerable input from the HSC population in the BM. In other words, T-cells show typical stem cell properties as they are able to dynamically balance survival, proliferation, and differentiation during homeostasis as well as after infection and/or injury. Also similar to other tissue stem cells, the fate of (naïve) T-cells is governed by their interactions with a complex niche environment, characterized by unique structural and chemical properties. In particular, T-cells interact via specific T-cell receptors (TCR) with complementary antigens, which are presented by other cell types and which provide necessary survival signals. Additionally to this clonotypic TCR-specific niche regulation, the size and the diversity of the T-cell repertoire is controlled by cytokine signaling, a regulatory mechanism that has been addressed by Megan Palmer. The presented data suggest that cytokine levels in normal mice maintain a homeostatic balance between heterogeneous T-cell populations, and the therapeutic addition or depletion of cytokines can be used to selectively expand certain T-cell subpopulations. Furthermore, it could be demonstrated that mild intrinsic heterogeneities in cytokine receptor levels can yield significant differences in cell proliferation and survival responses. These results clearly point out that niche-mediated control potentially involves different mechanisms (here: direct TCR-mediated cell–cell contact and cytokine signaling) and that niche heterogeneity might be an important regulatory feature (see below).

Intestinal crypt (epithelial stem cells)

In contrast to the HSC niche in the BM, epithelial stem cells in the intestine are clearly located within specific cup-like structures. These so called crypts consist of a polarized mono-layered epithelium and contain (in mice) about 235–250 cells [13]. Only about 150 of those cells, located in the lower part of the crypts, show proliferative activity and provide the source for the constant replacement of differentiated cells in the upper part of the crypts and on the so called villi on top of them [14,15]. As the crypts are maintained on a long time scale and as the proliferative capacity is restricted to the lower part, it is clear that the stem cells have to be located within this region [16]. It appears that stem cells give rise to Paneth cells, which contribute to forming the stem cell niche by providing a high level of Notch ligands.

[2] Previous *StemCellMathLab*'s were held in 2001, 2005, 2007 (Leipzig, Germany) and in 2008 (London, UK).

Furthermore, the intestinal stem cell niche is characterized by a high Wnt pathway activity that is probably induced by the high positive curvature in this region [17].

Lung (airway stem cells)

Lung stem cells appear to be restricted to specific anatomic sites throughout lungs, i.e. submucosal gland ducts, airway branch points, and bronchio-alveolar duct junctions. Those niches for lung stem cell show three typical characteristics that can also be found in other systems: they maintain the intrinsic properties of the stem cells themselves, they facilitate the interaction between stem cells with the local microenvironment, and they influence systemic or anatomical factors that regulate stem cell function. As summarized by Adam Giangreco, intrinsic properties of lung stem cells include the capability of significant proliferation and multipotent differentiation, but also pathogen and injury resistance through both intrinsic and extrinsic mechanisms. Extrinsic (i.e. niche mediated) cues include enhanced local cell adhesion (both cell–cell and cell–ECM) as well as (in some niches at least) intimate association with a neurosecretory epithelial population. All of these properties bear significant similarities to those of other epithelial stem cell niches, in particular intestinal crypts.

Although the above examples clearly show the highly variable appearance and the peculiarities of different niche systems, they also identify a number of common features shared by different stem cell niches:

- There are key signaling pathways that are shared among different tissues (e.g. Wnt/beta-catenin, Notch)
- For all described tissues, stem cells are supported by "helper" cells (e.g. osteoblasts for HSC, antigen-presenting cells for T-cells, paneth cells for epithelials stem cells, neuroepithelial bodies for lung stem cells)
- Intercellular adhesion (and enhanced integrin expression) is a common mechanism for localization and signaling among many stem cell types.

Regulatory components of the niche

The above outlined examples illustrate that niches can comprise various combinations of environmental cues to integratively yield a proper 'signaling network state' of the corresponding stem cells. Important examples of regulatory cues are growth factors and cytokines, such as Notch ligands, Wnt proteins, bone morphogenetic proteins, angiopoietin-like factors, or thrombopoietin (TPO). Those extrinsic factors should, however, not be regarded in isolation. They interact with a huge number of cell-intrinsic components such as cell-cycle regulators (e.g. p16, p18, p21, p53, Myc), transcription factors (e.g. Gata2, Hoxb4, Junb, Sox17, PU.1) or chromatin-associated factors (e.g. Ezh2, Bmi1, Mll, Rae28). As reported by Baiba Vilne and Robert Oostendorp most of these stem cell regulating factors were found by focusing on the steady state gene expression profile of either HSC or their microenvironment. Along those lines, Douglas Lauffenburger pointed out that an important portion of network cross-talk is realized extracellularly, via feedback cascades of autocrine/paracrine circuits, which can interact with the extracellular matrix and neighboring cells.

Particular signaling pathways that are discussed in the context of HSC niches are e.g. the Notch pathway [4], Ang1/Tie2 [18], SDF1/CXCR4 [19], TPO/Mpl [20] or SCF/c-Kit [21]. Despite initial reports claiming that beta-catenin, the key component of canonical Wnt signaling, is not necessary for hematopoiesis [22], a growing number of studies now indicate that Wnt signalling does affect HSC function [23–27]. As discussed by Cristina LoCelso, particularly the over-expression of the Wnt inhibitor Dkk1 in mouse osteoblasts resulted in impaired self-renewal and function of both endogenous and transplanted HSC [28], thus suggesting Wnt signaling level to be an important niche-related regulator element.

Another specific aspect of niche-related stem cell regulation is cell–cell and cell–ECM interaction. In particular for the hematopoietic system, different adhesion and ECM molecules such as cadherins, integrins, or osteopontin have been shown to regulate stem cell function [29–31]. However, as mentioned by a number of workshop participants (e.g. Tilo Pompe, Carsten Werner, Cristina LoCelso), even though the presence of specific stroma cells and ECM components inside the BM has been reported [32,33], their detailed spatial arrangement is still unclear. In this context, Martin Bornhaeuser explicitly referred to mesenchymal stem cells (MSC), which represent an integral part of the BM, environment as key components of the HSC niche. In particular, he highlighted the role of MSC in generating appropriate ECM structure. The presented data showed that both of two different types of ECM, generated either using MSC cultures fed with ascorbic acid (aaECM) or MSC subjected to osteogenic differentiation (osteoECM) allowed for a more than 4-fold expansion of HSC compared to less than 2-fold expansion on control ECM substrates. These observations suggest that ECM preparations derived from MSC might be useful to accomplish better expansion of MSC and HSC under defined culture conditions.

Beyond the activity of certain pathways and the presence of different cellular and ECM components, it is suggested that the specific spatial arrangement of cellular and ECM components is relevant, too. In particular, in vitro studies have elucidated the responsiveness of HSC and other stem cells to mechanical and spatial constraints of the local microenvironment, partly in synergism with growth factors and chemokines [34–38]. A prominent example has been presented by Peter Buske, Joerg Galle, and Markus Loeffler. Based on a model study, they showed that the assumption of Wnt-pathway induction by local positive curvature of the intestinal crypt epithelium is sufficient to consistently explain experimental results the crypt formation from single cells in an in vitro environment [17].

Often overlooked in considerations of tissue organization is the issue of metabolic compartmentalization as discussed by Michael Cross. The areas of the bone marrow in which HSC are found lie well separated from the nutrient supply, where a low oxygen tension may help to limit oxidative damage. Over and above this, however, it is possible that the limited availability of metabolites such as glucose and amino acids may also have a decisive influence on signaling within the niche, in this way tying stem cells to a controlled environment.

Stem cells and the functional definition of stem cells niches

As pointed out by Doug Lauffenburger, the stem cell niche should be defined with respect to the stem cell signaling network state associated with appropriate phenotypic, behavioral responses, such as self-renewal, death, differentiation, or migration. That means, defining stem cell niches inevitably requires the definition of stem cells, as their proper functional appearance ("functiotype") is used as the defining end-point. However, the functional definition of stem cells, as proposed e.g. by Potten, Loeffler and Roeder [39,40], may include the niche as a regulatory/instructive environment itself. Therefore, it seems appropriate to consider stem cells and stem cells niches as an inseparable pair: potential stem cells need a certain niche environment to actually express their "stemness" potential and stem cell niches manifest their stem cell support only if they are faced to potential stem cells. Along these lines, Markus Loeffler suggested the following functional definition:

> *"Stem cell niches of a particular tissue are (often heterogeneous) supportive environments for functionally undifferentiated stem cells. Niches are capable of maintaining, modulating and stabilizing the distribution of functional characteristics and phenotypic markers of undifferentiated cells on the population level (*i.e. *enabling and also*

constraining fluctuations). Niches are able to integrate a multitude of environmental cues in a tissue specific way, thereby (i) maintaining many of these cells in the undifferentiated state (maintenance), (ii) supporting continuous production of differentiated progeny functional for the tissue (regeneration), and (iii) providing robustness against and recovery from perturbation (protection)."

Although it became clear, that tissue-specific aspects do play an important role, this general (i.e. tissue independent) functional definition achieved accordance among all workshop participants. It summarizes the perception that there is no unique niche architecture but that there are generic principles of niche organization. Furthermore, there was consensus that for both, stem cell and stem cell niche characterization, the cell or even more general the tissue function has to be regarded as the gold standard. The functional aspects cannot be substituted by some (isolated) "omics" surrogates; although molecular signatures depict certain intracellular states, the resulting "functiotype" of the cell is the result of a more complex interaction, integrating molecular, cellular, extracellular, biomechanical and spatio-dimensional effects.

As pointed out by Peter Zandstra the niche can also be regarded as a regulator of stem cell numbers. Whereas niche signals support stem cell survival, limited niche "space" (defined physically or with respect to the accessibility of a certain signaling context) might lead to a limitation of stem cell numbers, an aspect that might be important to understand cancer development in the context of niche dysregulation. A related aspect that was raised several times during the workshop is the proliferative activity of stem cells with or without the effect of niche signals. Because stem cells are the source for a life-long maintenance of a particular tissue, it appears plausible that they should avoid as much error-prone activities as possible. Therefore, avoiding cell division could be considered as an important safety feature to prevent the occurrence of mutation events. In the hematopoietic system, the niche environment might induce a deeply quiescent state of stem cells. In contrast, the loss of niche regulation will lead to proliferative activity. This duality of proliferative activity vs. quiescence of HSC has been discussed by Marieke Essers. She presented data [41] clearly demonstrating that HSC can reversibly change between the two functional states: actively proliferating and dormant, a concept that had also been proposed on the basis of a mathematical model prediction by Roeder & Loeffler in 2002 [42] (Fig. 1).

Niche heterogeneity

A number of workshop participants referred to the potential heterogeneity of niches even within a particular tissue. Robert Oostendorp brought up the duality concept of a homeostatic vs. a regenerative niche in the hematopoietic system (Fig. 1). It assumes that HSC normally reside in an environment that induces proliferative quiescence or at least slow turnover. In contrast, damage signals may change ("alert") the niche context such that it now promotes increased proliferative activity. Whereas this concept suggests changing feedback signals within one particular niche context, several studies have suggested (at least) two different locations for HSC niches in the bone marrow: an osteoblast-related niche near the endosteum [4,10] and a vascular niche near bone marrow sinusoids [11]. Both regions are suggested to induce different functional behavior of HSC and/or the support of different HSC subpopulations. It is still unclear whether there is a regulated exchange of HSC between these two regions. However, as pointed out by Marieke Essers there is the hypothesis that those two niche environments are related to a different proliferative activity status of HSC and that the exchange of HSC between the osteoblastic and the vascular niche may depended on the systems state, i.e. homeostasis, hematopoietic stress, repair or recovery (Fig. 1).

Another more conceptual aspect of niche heterogeneity has been mentioned in the presentation of Ingo Roeder. As demonstrated by computer simulations, the assumption of heterogeneity in niche properties (i.e. the presence of niches with slightly different properties) is able to consistently explain experimentally observed phenomena of clonal dominance. In particular, clone specific affinities for different niche types will result in a limitation of the contribution of otherwise aggressively expanding stem cell clones, as observed e.g. in the context of gene therapeutic settings [43,44].

Also for the T-cell systems, heterogeneity of niches or niche signaling appears as an important structural principle. Megan Palmer presented experimental and modeling results showing how subtle heterogeneities in cytokine receptor expression can yield large variations in functional responses across T-cell populations. In turn, heterogeneity in receptor expression has an impact on the extent to which cells can affect their microenvironment by depleting limited homeostatic resources.

Summarizing, there was consensus that a considerable degree of heterogeneity in terms of stem cell functioning has to be generated for each tissue. Specifically, situations of development, homeostasis, and regeneration require differential stem cell functioning (possibly inducing different stem cell phenotypes). Whether this is achieved by different niche environments (e.g. osteoblastic vs. vascular for HSC), between which stem cells have to change, or whether niche signaling is continuously altered, could not be conclusively decided and might be different from tissue to tissue.

Niche (induced) dynamics

A phenomenon that is closely linked to niche heterogeneity is the dynamic change of niche properties or of niche signals over time. Both will potentially have an effect on stem cell functionality and there was consensus that (niche-induced) reversibility of (stem) cell properties exists and has to be considered as an important organizational feature.

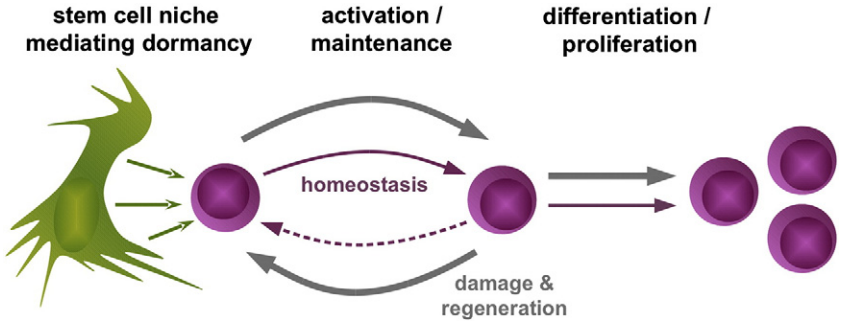

Fig. 1. Niche-mediated balance between stem cell dormancy and activation. The niche environment mediates stem cell quiescence, whereas loss of niche regulation leads to proliferative activity. This process is intensified in case of damage signals leading to regeneration (i.e. the signals "alert" the niche context). The figure is adapted from a scheme kindly provided by Robert Oostendorp.

An important aspect with respect to dynamic changes of cellular properties is the generation of heterogeneity in a population of cells. As demonstrated by Joerg Galle and Axel Krinner, different levels of stochasticity in the differentiation process of stem cells can account for typical degrees of heterogeneity as experimentally observed for mesenchymal stem cells [45,46]. In the context of environmental control of stem cell function and fate control, the niche has been discussed as a potential site that is able to modulate (e.g. to reduced) the degree of signaling variability. Thus, noise regulation is one potential mechanism of niche-mediated stem cell control.

Beyond niche-induced (stem cell) dynamics, it is possible that niche properties (e.g. niche-mediated signals) change over time. Although there was a broad consensus that a life cycle of niches should be expected in many cases, the particular nature of dynamic changes of niche composition and/or signaling is not understood. Nevertheless, most workshop participants believe that niche alterations occur during development, but also in response to the demand of the system, including the stem cells "inside" the niche, i.e. assuming dynamic mutual interactions. In this context, the hypothesis that niches cannot be regarded as pre-existing (static) entities but that they can be (dynamically) created, e.g. by the stem cells themselves, has been intensely discussed. Even though there is no particular experimental evidence for such a hypothesis so far, it would certainly fit into the perspective of stem cell–niche systems as being self-organized structures, as proposed e.g. by Markus Loeffler and Ingo Roeder [40,47]. An example, which might (at least indirectly) point to changing/adapting niche sizes, has been presented by Adam Giangreco for the lung system. Using aggregation chimera mice and a whole-lung imaging method, Giangreco demonstrated that the relative contribution of progenitor (Clara) cells and bronchiolar stem cells to epithelial maintenance and repair alternates between normal/ moderately injured airways and severe lung injury. In control or moderately injured mice, chimeric patches were small in size and not associated with previously described stem cell niches, suggesting that single, randomly distributed progenitor cells maintain normal epithelial homeostasis. In contrast, severe injury resulted in the generation of rare but large clonal cell patches that were exclusively associated with previously identified neuroepithelial body and bronchio-alveolar duct stem cell niches. These observations might be a hint for the use and/or generation of different niche environments depending on the system's needs.

Quantifying the niche

As discussed in the last section, both stem cells and stem cell niches require a functional definition. This viewpoint has two important implications: First, assessing stem cell niche interactions always comprises a dynamic component, because (stem cell–niche) function cannot be considered as a static entity but as a time-extended process. Second, a systemic understanding of either of the two components (i.e. stem cell and stem cell niches) requires the assessment of the complete system. In other words, stem cells and their niches should be studied together; an isolated assessment would not lead to a comprehensive understanding. Clearly, these insights lead to a number of questions about the experimental strategy for studying stem cell niche effects, for instance:

- (How) can we characterize the (potential) heterogeneity and/or the (potential) dynamic features of niches?
- Are classical in vitro and in vivo models suitable to address those features?
- Do we need synthetic/bioengineering approaches to overcome limitations in classical assay systems?
- Is in vivo imaging a reliable option for studying cellular behavior in situ?
- Can theoretical approaches foster the experimental assessment of stem cell niche interaction?

In the following we summarize those parts of the workshop, which aimed on answering the outlined questions.

In vitro niche-mimetics

As pointed out in the seminal presentation of Kateri Moore, the establishment of the long-term culture (LTC) system by Dexter et al. in the late 1970s [48] sparked interest in the in vitro hematopoietic microenvironment as a surrogate for studying the stem cell niche. This methodology allowed investigators to study the behavior of hematopoietic cells as they proliferated and differentiated in a culture dish. This was soon followed by the development of quantitative assays that allowed the assessment of the developmental potential of the cells as they were maintained in these cultures. The LTC-initiating cell assay, developed by Eaves and colleagues, combined LTC with the colony-forming cell assay [49] and was able to provide a quantitative estimate of primitive progenitors maintained in LTCs after extended time periods. Further on, Ploemacher's group developed another quantitative assay based on their observations of distinct types of hematopoietic colonies that developed under the stromal monolayer referred to as Cobblestone Area-Forming Cells (CAFC) [50].

In addition to these general assays many investigators derived stromal cell lines instead of using the heterogeneous, whole bone marrow derived monolayers in typical Dexter LTCs. A large number of stromal cell lines have been developed, allowing for more refined studies of the mechanisms that mediate stem cell support [51]. They provide useful means for mediating stem cell support of HSC in both gain and loss of function genomic screens targeted to the HSC as well as for the stromal cells themselves. In addition, genomic analyses determining the molecular cross-talk of both HSC and stromal cells during the in vitro culture period will provide new insights into the regulation of HSC self-renewal and differentiation that are not discernable by studies of both compartments individually. As pointed out during the workshop, gene expression is one characteristic that can be used to distinguished HSC supportive and non-supportive stroma cells. In this context Robert Oostendorp presented results, showing that deficiency of Sfrp1 [25] or Ptn (pleiotrophin) in stromal cells affects gene expression of cell cycle regulators in hematopoietic cells.

Bioengineering the niche

In vitro cultures using different types of niche-mimicking, stem cell supporting stroma cells are highly valuable in studying stem cell–niche interaction. However, they are not ideally suited to elucidate the extrinsic mechanisms of stem cell regulation because they show the usual restrictions of complex biological aggregates: individual components and different regulatory features can hardly be assessed and manipulated in a controlled manner. Here, bioengineering approaches offer fascinating new perspectives for quantitative studies and for disentangling niche components. Matthias Lutolf presented an overview on new technological achievement, ranging from microwell assays that allow for the high-throughput assessment of different niche factors to innovative microfluidics devices, such as interconnected micro-channels or systems that use microfluidically generated gradients of effector molecules. A detailed survey of these technologies can be obtained from Kobel and Lutolf (2010) [52].

As presented in the talk of Tilo Pompe, bioengineered, micrometer-sized silicone cavities covalently functionalized with proteins and glycoseaminoglycans of the ECM, are well-suited to investigate the influence of spatial restrictions and adhesive interactions on stem cell fate decisions. Analysis of human CD133+ HSC after culture on these surfaces revealed that proliferation and differentiation is

decreased when HSC are supported by substrates with small microcavities (collaboration work by Carsten Werner, Tilo Pompe and Martin Bornhaeuser). Moreover, single cell analysis of adherent cells showed decreased DNA synthesis and higher levels of HSC marker expression inside smaller cavities. The modulation of cytokine concentration highlighted the tight balance of adhesion-related signals and soluble cues acting on HSC fate decisions. The suppression of the adhesion-related proliferation and differentiation characteristics at high cytokine concentrations emphasized the importance of synergistic and antagonistic effects for HSC fate decisions. As the effects of spatial heterogeneity of niche factors must not be underappreciated, Peter Zandstra and Emanuel Nazareth presented an approach to overcome the difficulties to control and to quantify these factors. In particular, they introduced engineered microenvironments with "tunable" cell distributions allowing accounting for the fact that endogenous factors (such as secreted ligands) often scale with localized cell density. Using microcontact-printing technology this approach provides a means to rapidly and robustly study individual niche factors using a simple, scalable, and widely transferable method for controlling niche size, shape, and density of cells and effector molecules under fully defined media and substrate condition within a 96 well platform [53–55].

All these results demonstrate that bioengineered culture systems provide a collection of highly valuable tools to dissect stem cell–niche interactions. Specifically, they allow for manipulations and for the quantitative monitoring of stem cell response to e.g. varying ligand distributions and soluble growth factor concentrations within a defined two- or three-dimensional context. Although bioengineered environments do not completely resemble natural stem cell environments, they allow to explicitly verifying hypotheses generated in animal models, under strictly defined and controllable conditions. Moreover, as emphasized e.g. by Matthias Lutolf and Peter Zandstra, environment- and matrix-engineering approaches in the context of micron-scale systems allow for the combination of studying the effects of a huge number of different (combinations of) environmental stimuli with high-throughput measurements of the molecular response within (stem) cells.

Molecular high-throughput measurements

Time course measurements of the molecular repertoire of stem and niche cells in response to defined system perturbations are most informative for obtaining a dynamic view on the crucial regulatory mechanisms of stem cell–niche interactions. This implies that also with respect to molecular (high-throughput) measurements we have to shift the focus from looking at a static snapshot for a few conditions (e.g. differential gene expression of cells under two culture conditions) to a dynamic perspective monitoring the molecular response to (well-defined) interventions. Furthermore, as we are aiming at a systemic understanding, multiple levels of regulation, such as different "omics" measurements (e.g. genomics, proteomics, metabolomics) but also functional readouts have to be considered simultaneously. At this point it should clearly be emphasized that the integration of these qualitatively different types of information (including the different data types) is still a major scientific challenge, which definitely requires considerable methodological research and a collaborative effort of both experimental and theoretical scientists.

In order to understand, how the mutual interaction of HSC and their niche affects molecular expression pattern over time, the group of Robert Oostendorp performed a gene expression time series analysis of HSC and HSC-supporting UG26-1B6 stromal cells at 3 different time points of co-culture. As presented by Baiba Vilne, a gene ontology (GO) category enrichment analyses revealed changes in metabolism, transcription, regulation of chromatin structure and cell cycle in both cell populations over time, pointing to the complex interaction of different extrinsic-cues with cell-intrinsic pathways and with transcriptional regulation in HSC and vice versa. As changes in cell fate occur over time and as they are mediated by changes in regulatory networks, it is necessary to measure network dynamics in a temporally extended process during the course of differentiation. One suitable way to study this is the use of controlled perturbations. Along these lines Ihor Lemischka presented data obtained from a large-scale RNAi screen. Using bioinformatic tools, clusters of genes could be identified that respond similarly to a certain perturbation induced by a specific gene knock-down. Moreover, as the study comprised epigenomic, genomic, and proteomic data simultaneously, it was possible to study the mode of action and/or the target mechanisms of a certain perturbation [56]. The study clearly showed that there are considerable differences in the responses obtained at the different regulatory levels, therefore demonstrating the necessity to pursue multi-level studies instead of focusing on gene expression alone. In the context of interpreting the results of perturbation or intervention experiments Douglas Lauffenburger explicitly pointed to the importance of dose–response profiles. Due to frequently occurring non-linearities in dose–response characteristics, the same type of perturbation/ intervention might result in qualitatively different outcomes on the molecular but also on the cellular and tissue level depending on the quantitative degree of the perturbation/ intervention.

As pointed out before, heterogeneity of stem and niche cells should be considered as a potentially important regulatory feature to cover a wide and robust spectrum of stem cell characteristics. In order to assess this population-inherent heterogeneity on the molecular level one has to shift the focus from measuring average expression levels in cell populations towards a single cell resolution. Such approaches allow for the quantification of distributions of expression levels rather than obtaining a single average value. One way to address molecular expression on the single cell level is the combination of gene or protein expression measurements with imaging technologies, which will be discussed in the next paragraph.

Imaging technologies and single cell tracking

High-content imaging is a powerful tool to measure single cell protein and potentially mRNA expression via fluorescent in situ hybridization. The previously mentioned micro-contact printing technology (presented by Emanuel Nazareth) allows quantifying protein expression levels and subcellular localization for each individual cell within a cell colony. Furthermore, spatial distribution of expression within artificially modeled niches can be elucidated (radial trends, correlations with density, etc.) [57].

In a more general context, Timm Schroeder stressed the importance of analyzing stem cell behavior – including their interaction with their niches – continuously, long-term, and at the single cell level. He discussed how limitations of the currently existing imaging modalities prevent observation of single mammalian cell behavior over many days. Novel bioimaging and cell tracking approaches were presented allowing to continuous quantify cellular behavior under defined culture conditions for periods up to several days or even weeks. These approaches were used to address long standing questions in the field of HSC biology, including proofing the existence of hemogenic endothelium [58] and the instructive action of cytokines on blood lineage choice [59]. Novel developments also facilitate the continuous quantification of transcription factor protein levels in living cells to detect changes in cell-intrinsic molecular states in blood stem and progenitor cells. Similar image analysis techniques that allow reconstructing cellular genealogies (i.e. cell lineages) have been presented by Nico Scherf. He demonstrated a new image processing method that is able to recognize HSC within specific bioengineered culture systems, to track these cells automatically, and to reconstruct the genealogies of (almost) all cells in the culture for a total duration of about 4 days (unpublished data). The presented algorithm

furthermore allows to automatically quantify cellular characteristics, such as size, shape, location, migration velocity or contact with other cells. As the presented technology is able to correlate those cell-specific parameters with the genealogical relations it provides an amazing, novel perspective for analyzing the effects of niche components on HSC behavior, e.g. with respect to migration and/or attraction and repulsion by other cells.

Although these results demonstrate that there are already suitable technologies for single cell tracking available, it should also be noted that those approaches are mainly restricted to the in vitro situation. Tracking of cell behavior in vivo is still a major experimental challenge. Shahragim Tajbakhsh presented genetic models to investigate single cell behavior in tissue explant culture systems. Using clonally marked cells within embryonic structures called somites, which contain the stem cells for multiple lineages including muscle, they were able to follow cell divisions for at least one day [60]. Other tools including a Numb-GFP transgenic mouse are applied to follow symmetric and asymmetric cell divisions in the somites. These genetic tools are coupled with live video-microscopy and cell tracking to follow stem cells divisions and expansion of the population [61]. Shahragim Tajbakhsh also presented data on filming cell behavior in explant cultures of skeletal muscle during regeneration. In such regeneration models, in which the niche is destroyed but reinstated during re-establishment of homeostasis, the questions can be addressed "what is the niche in skeletal muscle and is it limiting in quantity." If the entire differentiated myofibre can act as a niche with the basement membrane ensheathing the muscle stem cell, this would not be limiting and it raises the question if the niche is an already defined anatomical location, or if the niche arises from a mutual interaction between the muscle stem cell and its environment [60]. Currently, both possibilities are being considered. A different in vivo imaging approach was presented by Cristina LoCelso. She demonstrated how in vivo confocal/two-photon microscopy of mouse bone marrow calvarium can be applied to investigate the relationship between transplanted HSC and their local microenvironment at single cell resolution, in three dimensions and in real time immediately after transplantation and until few days later [12]. Based on the imaging data it could be shown that long-term repopulating HSC are the most selective cells to localize near osteoblasts and that there may be a correlation between HSC function and their localization within the bone marrow microenvironment.

Modeling the niche

Building upon the observations and results discussed earlier in this report, the stem cell niche comprises various combinations of environmental cues leading to an adequate 'signaling network state' of stem cells. Therefore, as pointed out by Douglas Lauffenburger, the niche should be defined with respect to stem cell signaling network states associated with appropriate functional responses of these cells. This perception of stem cell–niche interaction as the result of multivariate signal-response relationships has implications for the theoretical and modeling analyses: It requires the use of conceptually different approaches that need to be integrated (see Fig. 2). In his talk, Douglas Lauffenburger presented an overview of suitable modeling techniques that are able to address important aspects of these different regulatory levels. Particularly he focused on two types of modeling techniques: Partial Least Square (PLS) [62] and Constrained Fuzzy Logic (cFL) [63,64] models. This type of analysis allows for identifying potential interactions of effector variables, as demonstrated for the example of epidermal growth factor (EGF): While increasing levels of EGF have an anti-apoptotic effect in combination with high levels of tumor necrosis factor (TNF), the same signal leads to a pro-apoptotic effect at low TNF concentrations. This example demonstrates that identical ligand "cues" can induce diverse phenotypic responses in different contexts. This is an important insight with respect to the analysis of niche–mediated regulatory "cues": The same molecule/signal might have a different effect in a certain (niche) context even though it might be known for another effect in a "non-niche" context. Beyond the identification of effector interactions, PLS models are suitable to reduce the dimensionality of the multitude of potential influences, e.g. by using a principal component analysis (PCA). Using weighted linear combinations of different effector signals, PCA methods allow for data reduction to canonical "signaling eigenvectors" or "eigengenes" that restrict the description to major effect combinations, therefore, providing insights into relevant components of the cells' signaling network information processing. To link these signal-response relationships to the underlying network logics, another modeling approach, namely constrained Fuzzy Logics has been proposed by Douglas Lauffenburger [65]. This model class can be used to convert (empirically derived) qualitative knowledge on networks of signaling molecules or regulatory components into a quantitative, computable model.

The workshop discussions clearly pointed out that the identification of relevant network motifs and the study of possible resulting network dynamics requires moving the experimental strategy from a static snapshots perspective (e.g. expression status in a cell population at one point in time) towards a dynamic analysis of molecular responses over time, preferentially following a number of defined perturbations. Such an approach that combines systematic RNAi-based perturbation experiments, complemented by parallel time series measurements of gene and protein expression as well as of histone acetylation and chromatin-bound RNA polymerase II as markers for epigenetic regulation has been presented by Ihor

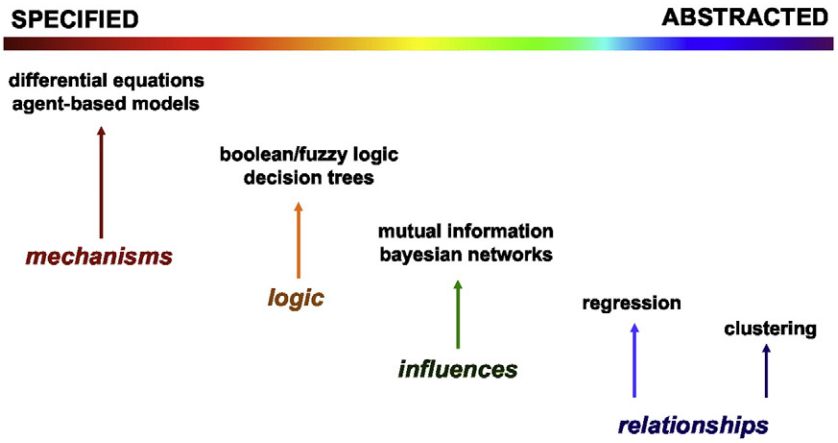

Fig. 2. Spectrum of computational/modeling methods. The sketch provides a structured overview about different (statistical and dynamical) modeling methods and their suitability for different levels of description. The figure is adapted from a scheme kindly provided by Douglas Lauffenburger.

Lemischka [56]. Specifically, Ihor Lemischka introduced a bioinformatic tool (GATE - Grid Analysis of Time series Expression [66]) that allows visualizing and analyzing time series measurements of expression data. Particularly, the tool considers the dynamical behavior of correlated clusters of genes or proteins, thus revealing dynamic patterns in the regulatory response of cells. In contrast to this top-down approach, which attempts to reconstruct networks from high-dimensional experimental 'omics'-data using reverse engineering techniques, Joerg Galle presented a bottom-up strategy. It uses a simple model of transcriptional regulation based on thermodynamic rules in the context of random genomes to study the response of the global expression pattern to changing properties of basal regulatory building blocks. The presented model predicts single-peaked distributions of expression values of which the flanks decay according to power laws. Analyzing experimental data, it could be demonstrated that the study of such global expression pattern provides valuable information about transcriptional regulation complementing conventional searches for differentially expressed single genes [67].

To link results regarding network structure (topology) and generic regulatory rules to an understanding of functional cellular responses (such as differentiation or cycling activity) one has to investigate the dynamic properties resulting from those rules and acting within the identified network structure. One possible way is the application of differential equation models as presented by Ihor Lemischka. Using stability and bifurcation analyses, these models allow studying dynamic properties of networks with respect to changing parameters values. As the parameters values can potentially be linked to the above described "signaling eigenvectors" (which can be statistically estimated from data), this model type might help to integrate environmental effects (e.g. receptor/ligand interactions) with insights on the intracellular regulatory network structure. The same model class (i.e. differential equation models) has been applied by Richard van der Wath to study cell kinetics of HSC. Using an ordinary differential equation, he showed that experimental results on label dilution [68] are not consistent with a homogenous population of HSC. Instead, the mathematical results demonstrate that one has to assume (at least) two subpopulations of HSC: a deeply quiescent and a slowly cycling one [69]. Instead of interpreting deeply quiescent and a slowly cycling HSC as different HSC subpopulations, Ingmar Glauche presented a model analysis that consistently explains the experimental observations on the basis of a dynamically stabilized population of HSC, which considers two functional states of these cells. Whereas HSC under the influence of niche signals are assumed to switch off their proliferative activity, they are able to be reversibly activated into an actively proliferating state [70]. This concept of a (niche) context-dependent, dynamically stabilized population of HSC [42] has been implemented as a single cell (agent)-based model, which allows to explicitly account for the combination of regulatory effects of different scales, such as molecular networks within individual cell, the interaction of many individual cells, as well as spatial or context-dependent constraints. Another example for the predictive potential of single cell-based modeling approaches has been presented by Peter Buske, Joerg Galle and Markus Loeffler for the effect of molecular disturbances within individual (stem) cells (e.g. the knock-down/overexpression of Wnt) on the spatio-temporal organization of the intestinal crypt [17].

The fact that feedback regulation is ubiquitous in biological systems and is an important component of the natural organization of biological networks has also been discussed by Geoff Clarke and Peter Zandstra. Modeling the behavior of intercellular regulatory networks can be used as a powerful tool to better understand cell fate decisions during in vitro human blood SC propagation [71]. The presented model links functional cellular assays to specific model outputs, defines cell level kinetic parameters such as cell cycle rates and self-renewal probabilities as functions of culture variables, and simulates feedback regulation using cell–cell interaction networks. Computational analyses of the system dynamics indicate that soluble non-cell autonomous parameters (cell–cell interactions) are dominant factors controlling SC growth.

Adding to these specific modeling approaches, the issues of noise and stochasticity were vividly discussed during the workshop. It appeared that these terms are used rather loosely although their meaning should be made very clear. In the context discussed here, noise does not necessarily refer to an unwanted or even unpredictable signal. Rather it is used in its physical meaning, i.e. it represents a random process describing the overlay of specific regulatory signals by other, unspecific signals. Also, stochasticity or randomness does not necessarily imply complete unpredictability. Depending on the particular type of the underlying random process (specifically of its variability and correlation structure), predictions can be obtained in terms of an ensemble average within a certain confidence range, i.e. linked to a certain error probability.

Summary

The workshop outlined a number of important, general insights that attained common consent among the participants. First of all, there was consensus that, similarly to stem cells themselves, also stem cell niches should be defined functionally. In this sense, stem cell niches represent microenvironments that functionally support stem cells, by maintaining (many of) these cells in an undifferentiated state, by supporting continuous production of differentiated progeny functional for the tissue, and by providing robustness against and recovery from perturbation. Although stem cell niches appear in different, tissue-specific flavors, their function seems always to be based on a number of general regulatory principles. In particular, stem cell niches affect the number of tissue stem cells by controlling proliferative activity and differentiation propensity. In most cases the niche can directly be associated with particular anatomical regions, which, due to the local restriction, imply a competition of stem cell (clones) for (the limited) niche resources. Although the niche is linked to a certain anatomical site, it should not be considered as a static structure. Many of the workshop participants consider the niche as a dynamic system that integrates specific extracellular matrix and stroma components, growth factors and signaling molecules, metabolic cues as well as biomechanical features and spatial constraints. Furthermore, cell–cell interactions and, therefore, also the stem cells itself have to be considered as integral parts of this dynamical system. As a direct implication of such a dynamic perspective, stem cells and stem cell niches should be considered as an inseparable pair: Although stem cells and other niche components could formally be separated, their actual function (or, in other words, their functional potential) changes if either component is altered. Along these lines, "stemness", i.e. stem cell potential, must not be perceived as a purely intrinsic property of (stem) cells, but as originating from a reciprocal interaction of stem cells and their microenvironment. Another important aspect of the workshop discussions was heterogeneity. There is accumulating evidence in many tissues that there is a variety of different niches, responsible for supporting stem cells in different systemic states, such as homeostasis, development, or regeneration after injury. This, together with the perception that stem cell–niche systems need to be perceived as dynamically changing systems, leads to the conclusion that heterogeneity of niche locations, of niche signals or of (induced) stem cell properties is an important regulatory feature requiring both experimental and conceptual investigation. In this respect it is insufficient to solely concentrate on a certain function of a particular niche component in a particular (experimental) situation. A specific regulator (e.g. a specific molecule) might have completely different effects if considered in a different context. Understanding the regulatory mechanisms of dynamics systems, such as the stem cell niche, requires a more generic, abstract view that is not centered around particular regulator molecules, but focuses on functional principles.

Such a view can benefit from the application of modeling approaches. In particular, mathematical models can help to understand the role of regulatory principles such as heterogeneity, nonlinearities or scale-bridging interactions in the regulation of stem cell dynamics. To study dynamical systems different theoretical approaches are available (Fig. 2). With respect to achieving a systemic understanding of stem cell (niche) organization, it will be essential that the applied models consider different levels of regulation, aspects of context dependency, and the potential impact of heterogeneity. Beside an identification and quantitative description of the molecular regulation networks, this requires the application of scale-bridging approaches, which are able to account for intracellular but also for intercellular regulations and which do account for different levels of heterogeneity.

Clearly, mathematical approaches need reliable experimental data for building and for validating the models. To be able to quantitatively account for the dynamic nature of the systems, experimental strategies need to include time series observations with and without controlled perturbations. To adequately represent the above describe aspects of heterogeneity, it would be most informative to focus on single cell rather than cell population readouts. Here, in particular the use of new technologies is highly valuable, such as bioengineered niche environments, single cell imaging and tracking approaches as well as molecular screens and RNAi perturbation strategies at the single cell level. Detailed quantitative characterization of cell responses under defined in vitro conditions can be used to deconvolute in vivo system behavior and to characterize effective in vivo environments by using the cell state upon isolation as an environmental probe. To demonstrate the biological relevance of identified regulatory mechanisms the experiments in “artifical” in vitro systems have of course to be complemented by in vivo experiments. Although still in its infancy, in vivo imaging technologies will soon become a relevant and profound tool for an in situ quantification of stem cell–niche interactions.

The workshop discussion pointed out that a comprehensive understanding of the highly complex stem cell–niche system can best be achieved by an interdisciplinary alliance of biologists, bioengineers, bio-physicists, mathematicians, and computer scientists. In this respect the 5th StemCellMathLab was an encouraging and very successful first step.

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