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Do Stem Cells Play Dice?

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The debate surrounding the issue of whether or not hematopoietic stem cell commitment and differentiation are orchestrated in a cell-intrinsic or cell-extrinsic fashion is in many ways akin to the nature versus nurture debate that typifies discussions of human personal potentials. We have published data that can be interpreted as supporting both hypotheses. Simply put, the question is this: is unilineage commitment the result of a cell-autonomous or internally driven program or rather is it the consequence of a cell responding to an external, i.e., environmentally imposed, agenda?

In the former case, stochastic processes are often invoked as instigators of lineage decisions. In instructive, or so-called deterministic schemes, cell-cell interactions or diffusible signals dictate cell fate decisions. Much attention has revolved around the role played by hematopoietic growth factors in this process: do they play an instructive, i.e., a deterministic role, or are they simply permissive or selective, i.e., allow the survival and proliferation of independently committed cells? Certainly, experiments using multipotent cell lines in vitro suggest that the addition of exogenous growth factors is essential for the survival and proliferation of the cells but not for the lineage commitment or subsequent differentiation and development into mature cells. However, it has been argued that cultured cell lines, albeit with a normal karyotype, growth factor dependence, and ability to undergo multilineage differentiation, may not adequately reflect what happens with freshly isolated normal cells. To address this criticism, the intuitive experiment of adding cytokines, alone or in combination, to a population of multipotent cells in vitro and recording lineage output would, on the face of it, be a simple way of distinguishing between stochastic versus deterministic control of differentiation. Unfortunately, this experiment is flawed. For example, the elicitation of granulocytes from a pool of multipotent cells by the addition of G-CSF would seem to argue for an instructive role for G-CSF. However, the result could equally well be explained by suggesting that G-CSF is selecting for the survival and proliferation of a subpopulation of cells that have already been programmed for neutrophil development (by cell-intrinsic/ stochastic means), with the other cells, with different lineage potential, either remaining undeveloped/unexpanded or simply dying. In this respect, in vivo experiments with all the currently assessable knock-out mice lacking growth factors and/or their receptors have strongly suggested that growth factors are not essential for lineage determination. For example, mice lacking...
GM-CSF show no obvious deficiencies in the production of myeloid progenitor cells in the bone marrow; mice lacking G-CSF show a reduced but still substantial production of neutrophils; and the erythropoietin (Epo) and Epo receptor knock-out mice still produce near-normal levels of lineage-committed erythroid precursor cells.6 Although these data do not rule out the possibility that these growth factors can be involved in lineage commitment decisions, the data do show that lineage commitment decisions can be made in the absence of these growth factors.

Further information has come from molecular studies of the cytokine receptors themselves. While maintaining specific private ligand binding domains, many receptors share common or public signaling domains. Delivering a deterministic signal through common or shared subunits poses something of a mechanistic conundrum and raises the familiar specter of redundancy. Receptors that deliver signals through homodimerization perhaps offer more potential for instructive signaling, but even these appear to activate, if not identical, than very similar or highly overlapping, downstream signaling pathways. Noteworthy here, however, and potentially supportive of an instructive role is the molecular delineation within the cytoplasmic signaling domain of the G-CSF receptor of separable proliferation and differentiation-associated regions.7,8

Attention has also focused on the expression pattern of the cytokine receptors, particularly within multipotent cells: a cytokine-mediated instructive signal cannot be delivered to a multipotent cell that lacks a receptor for it! Most recently, McKinstrey et al9 have examined receptor distribution on purified stem and progenitor cells using radiolabeled cytokine ligands as probes. Heterogeneity was observed both in terms of the percentage of the population expressing receptors and the numbers of receptors expressed by labeled cells. Expression levels were in many cases low (<100 receptors/cell). Strikingly, the expression of receptors for GM-CSF (GM-CSFR) and M-CSF (M-CSFR) on stem cells was below the level of the expression of receptors for GM-CSF (GM-CSFR) and levels were in many cases low (numbers of receptors expressed by labeled cells). Expression pattern bias were detected, arguing against a deterministic role for growth factors in lineage commitment.

Because the signals themselves often activate similar if not identical pathways, different outcomes could presumably be dependent on different cellular perceptions or interpretations, ie, the notion of context-dependent interpretation or cellular history. An example comes from studies of the G-CSFR in which ectopic expression and activation in hematopoietic cell lines can lead to a proliferative response if expressed in BAF3 cells, but induce differentiation if transfected into L-GM cells.7

What then might likely constitute context at the molecular level? The status of cross-regulatory or cross-talking signaling pathways undoubtedly factor into the mix, but a more significant component is likely provided by the transcription factor profiles of individual multipotent cells. Many lineage-restricted or lineage-affiliated transcription factors have been described and their role in lineage specification addressed, primarily through gene targeting in mice.8 Strikingly, there is not a yet a single example of a resulting lineage-specific ablation at the level of commitment. That is not to say that the hematopoietic systems of these animals are not in many instances grossly affected with major defects in mature blood cell development. For example, GATA-1 knockout mice are severely anemic as a result of a failure in erythroid maturation, although erythroid commitment (ie, progenitor numbers) is largely unimpaired. As with growth factor receptors, the issue of overlapping functions and expression patterns as well as compensating pathways may well as yet be obscuring authentic roles for some of these
transcription factors in commitment. The next generation of multiple and conditional knockout mice will undoubtedly help to resolve this.

Ectopic master gene experiments based on the myoD paradigm in muscle have been conducted with some success, although primarily in chicken cells. Kulessa et al\textsuperscript{19} have shown that ectopic expression of individual transcription factors can reprogram the lineage output of myb-ets–transformed chicken progenitor cells. GATA-1 is a case in point, and here quantitatively different levels of GATA-1 are associated with different lineage outcomes suggesting that threshold effects may be in play.\textsuperscript{19} Importantly, their work, as well as our own, also emphasizes the role of negative regulation in lineage specification.\textsuperscript{20-22} Rejection of other lineage options is in some sense a corollary of unilineage commitment, and the observation that transcription factors can promote one lineage program while simultaneously and actively repressing another squares well with this. The recent demonstration that Ikaros may function to sequester loci into transcriptionally repressive regions of the nucleus may also be important in this regard.\textsuperscript{23}

Because lineage determination ultimately results from the collective assembly of stable transcriptional complexes at lineage affiliated loci such as $\beta$-globin, myeloperoxidase (mpo), Ig, etc, it is relatively easy to see how transcription factors must play a crucial role in differentiation through activation of a host of lineage-affiliated or lineage-specific target genes. It is perhaps less obvious to imagine how the ball starts rolling, and indeed rolling in any one particular direction.

Some intriguing evidence has recently come to light from the detailed molecular analysis of multipotent stem and progenitor cells. Studies of chromatin structure of multipotent cells have shown that a number of lineage-affiliated genes (globin, mpo, IgH, CD3\delta) have accessible control regions (enhancers/LCRs) before unilineage commitment.\textsuperscript{24,25} This accessibility is reflected in the fact that low-level, possibly spontaneous, transcription can be detected from a number of these genes and that RT-PCR analysis of a variety of differently purified multipotent cells has demonstrated expression of a variety of different lineage-affiliated transcription factors and cytokine receptors.\textsuperscript{11} Furthermore, analysis of single cells has shown that many different lineage-affiliated components can be expressed in the same cell, although heterogeneity in specific patterns or profiles of expression was observed. These data are consistent with a model of lineage specification of type shown in Fig 1, in which low level multilineage gene activity establishes a ground state or level of noise from which regulatory networks can start to build and be amplified or diminished either through quasirandom or stochastic changes in the components (ie, spontaneous transcription of an accessible activator or repressor) or through positive/negative reinforcement through extracellular signalling via stochastically expressed receptor molecules. Such a model incorporates both cell-extrinsic and cell-intrinsic components and may critically not be dependent on any one specific component to instigate a commitment decision.

Finally, it should be noted that what is true for multipotent or stem cells, which must service a lifetime's supply of blood cells, may not hold for more developmentally restricted progenitor cells. If differentiation of stem cells was simply instructive, it is difficult to imagine how stem cell homeostasis can be maintained in the face of competing demands for one of the stem cell lineages in response to physiological insults. Notably, cell proliferation in the bone marrow must be able to respond rapidly and efficiently to bleeding, infection, etc, and here perhaps it is more reasonable to propose that growth factors
have a role to play in the differentiation of lineage-restricted progenitor cells such as the bipotent granulocyte-macrophage colony-forming cells (GM-CFC). Highly enriched GM-CFC develop into granulocytes in the presence of SCF or G-CSF and into macrophages if cultured in M-CSF. In a series of studies aimed at investigating the molecular mechanisms underlying lineage restriction in the presence of these growth factors, we showed that macrophage development is associated with translocation of PKCα from the cytoplasm to the nucleus. Indeed, if GM-CFC are grown in the presence of agents that translocate PKCα to the nucleus (e.g., TPA and IL-4) even in presence of growth factors that usually promote granulocytic development, macrophages develop.26,27 Similarly, using clonal analysis of paired daughter cells of GM-CFC, Metcalf and Burgess28 showed that GM-CSF and M-CSF can apparently irreversibly commit cells to different lineages. Thus, certain of the growth factors may be able to play a role in lineage determination of these bipotent GM-CFC via known regulatory mechanisms.

In conclusion, our current hypothesis is that primitive cell differentiation involves mainly stochastic processes, but, as the cell becomes more lineage restricted, deterministic processes appear to be more relevant as the cell has to respond to immediate changes in the environment. Ultimately, one cannot help feeling that the all or none instructive versus selective debate is no longer the question. It is clearly important now to piece together the different molecular components, cell-intrinsic and cell-extrinsic, and understand the circuitry of their interactions. The precise starting point of a lineage-determining loop is probably not important. Indeed, it may vary between cells and therefore be unknowable. It is the chicken and the egg scenario all over again and who really cares which came first—apart from the chicken!

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