and enrich tissue-specific progenitors which may prove to be MSC-like cells. Such reagents have the potential to accelerate the pace for the translation of MSC-like cells to the clinic, as well as to advance our understanding of their basic science at the mechanistic level. Additionally, phage display may yield peptides suitable for targeted manipulation and/or selective homing of MSC-like cells in situ. Indeed, Kolonin and colleagues have employed such approaches to selectively ablate adipose tissue in vivo (Kolonin et al., 2004).

While resistin has been studied for over a decade, its receptor and signal transduction mechanism remains an active area of investigation (Schwartz and Lazar, 2011). Few studies have reported evidence of a resistin receptor (Schwartz and Lazar, 2011). The current work provides novel evidence that resistin interacts directly with decorin. It remains to be seen if and how decorin mediates resistin signal transduction in multiple tissues. Of course, there are always caveats. For example, there are species-specific differences in the expression and functionality of resistin (Schwartz and Lazar, 2011). While resistin is associated with adipocytes in the mouse, human macrophages also express this protein, so the biology of the WAT7/decorin interaction in the mouse may not mimic that in man (Schwartz and Lazar, 2011). Still, such minor points should not detract from the bigger story, namely, that phage display methods have emerged as an unbiased in vivo discovery tool for the characterization and identification of novel surface markers and signal transduction pathways for stem cells. This method has the potential to shed new light on our understanding of how stem cells in solid organs interact with their microenvironmental niche in situ. The next challenge will be for other stem cell laboratories to reproduce and expand on the UT-Houston team's exciting approach.

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Wnt to Notch Relay Signaling Induces Definitive Hematopoiesis

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The molecular mechanisms specifying hematopoietic stem cells (HSCs) in the vertebrate embryo remain poorly understood. Recently in *Nature*, Traver and colleagues demonstrate that timed wnt to Notch relay signaling across multiple cell types serves as an early upstream mechanism of HSC induction in zebrafish (Clements et al., 2011).

Every second of our life, millions of blood cells have to be replaced to maintain our blood system. All these cells are ultimately produced from hematopoietic stem cells (HSCs), which have the capacity to give rise to all blood cell types and to self-renew in order to maintain HSC numbers life-long. Due to their fascinating ability to repopulate the entire blood system of a recipient organism upon simple transplantation, HSCs have successfully been used for regenerative cell therapy for decades. However, the low number of clinically available HSCs remains a major restriction for their application. Novel approaches for their expansion or de novo generation in vitro would therefore have a huge clinical impact.

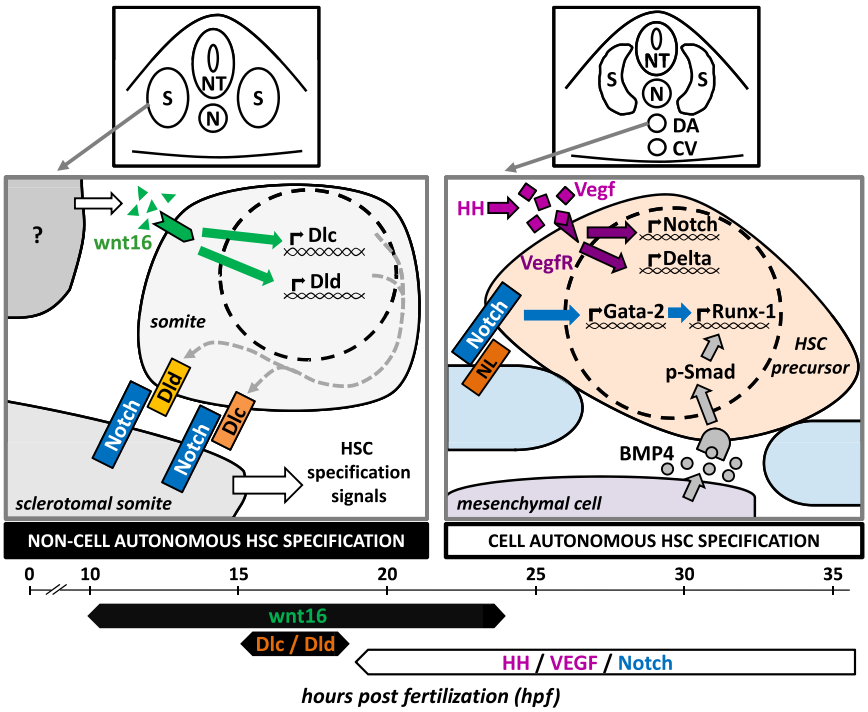

Figure 1. Signaling Pathways Involved in Specification of Vertebrate Definitive Hematopoiesis

Non-cell-autonomous signaling in somites via wnt16/Notch pathways is first required before the formation of the dorsal aorta (left). HSC specification is later induced by cell-autonomous signaling including HH/VEGF/Notch in the ventral wall of the dorsal aorta (right). S, somite; NT, neural tube; N, notochord; DA, dorsal aorta; CV, cardinal vein; HH, hedgehog; NL, Notch ligand.

However, despite decades of research, HSC generation by in vitro differentiation from pluripotent cells such as embryonic stem cells (ESCs) or patient-specific induced pluripotent stem cells, or by direct reprogramming from somatic cells, has not been accomplished. Improved understanding of HSC specification from their embryonic progenitors therefore is not only of highest scientific interest, but is also a crucial prerequisite for the development of novel therapies.

After decades-long disputes about the cellular source of the first embryonic blood cells, bioimaging approaches recently allowed the observation of endothelial cells differentiating into blood cells at the single-cell level (Eilken et al., 2009; Bertrand et al., 2010), demonstrating the existence of hemogenic endothelium as a source of embryonic hematopoiesis. Knowledge of the cell type from which blood originates is an important prerequisite for studying the involved molecular mechanisms. However, while numerous molecular pathways in different cell types have been implicated in controlling blood specification, their exact temporal and spatial integration remain poorly understood (Ciau-Uitz et al., 2010; Paik and Zon, 2010).

After analyzing embryonic hematopoiesis in zebrafish, Clements and colleagues now introduce wnt signaling as a novel player in induction of definitive hematopoiesis (Figure 1; Clements et al., 2011). Zebrafish is an excellent model system for analyzing developmental hematopoiesis. In addition to easy in vivo imaging of transparent embryos developing ex utero (Bertrand et al., 2010) and the availability of very efficient genetic tools, many required methodologies for analyzing hematopoiesis have also been developed for zebrafish (Paik and Zon, 2010). As in mammals, zebrafish hematopoiesis has two distinct waves. The "primitive" HSC-independent wave from about 12 hr postfertilization (hpf) generates a transient population of differentiated embryonic effector cells, such as erythrocytes. Later, the therapeutically relevant "definitive" hematopoietic cells are generated, which maintain all blood cells throughout adult life. Definitive HSCs emerge at around 30 hpf from the ventral wall of the dorsal aorta. Integration of Hedgehog (HH), vascular endothelial growth factor (VEGF), bone morphogenic protein (BMP), and Notch signaling has been shown to be involved in the generation of definitive hematopoiesis in vertebrates (Figure 1; Cerdan and Bhatia, 2010; Ciau-Uitz et al., 2010; Paik and Zon, 2010). Signals from these pathways drive expression of required transcription factors including Runx1, which is essential for the specification of the signal-receiving cells as HSCs.

Clements and colleagues have now identified a zebrafish wnt16 ortholog, which is expressed at around the time and sites of HSC specification. β-catenin-independent wnt16 signaling initiates Notch signaling by inducing expression of the deltaC (Dlc) and deltaD (Dld) Notch ligands. Importantly, these Dlc/Dld-mediated Notch signals (Figure 1) are different from the Notch signals previously described to be involved in the cell-autonomous HH/VEGF/Notch signaling required for HSC specification (Figure 1). Wnt16 signaling is required well before the actual HSCs are specified, and starts a cascade of signals through different cell types including different somite cell types, and later probably mesenchymal cells underlying the aorta and endothelial cells. Interestingly, both Dlc and Dld expression are simultaneously required at this early step to initiate the relay signaling cascade leading to HSC specification.

Notch and wnt signaling pathways control a multitude of developmental and adult cell fate decisions (Cerdan and Bhatia, 2010). They are also very complex and notoriously difficult to analyze. Due to the many ligands and receptors with unclear interactions, and many potential extracellular and intracellular modifiers, these pathways and their functions in controlling cell fate remain poorly understood. For wnt, there are two main intracellular signal transduction pathways, the β-catenin/TCF/LEF-dependent and the β-catenin-independent—usually referred to as canonical and noncanonical pathways, respectively. However, their clear separation, the involvement of specific ligands and receptors, and their independence remains disputed. The role of Notch signaling in inducing definitive hematopoiesis is well accepted. In contrast, its role in adult hematopoietic stem and progenitor

cells remains highly controversial, with different studies postulating an important function in HSCs, or no role whatsoever (Cerdan and Bhatia, 2010; Maillard et al., 2008). The role of wnt signaling in HSCs is equally unclear, with claims of a role in controlling hematopoietic stem and progenitor fates, or that it is dispensable in HSCs (Malhotra and Kincade, 2009; Cerdan and Bhatia, 2010). It will be interesting to see if these novel insights into the role of wnt signaling in HSC specification can also help to clarify potential roles in the complex niches of adult HSCs.

Although the current study includes some indications of the cellular source of the wnt16, precise identification of the specific wnt16-producing cell type, and of the molecular control of its expression, will be necessary to comprehensively unravel blood specification. The simultaneous requirement of two independent Notch ligands is puzzling and of wide interest. Part of the mechanisms behind this synergy has probably just been described in another recent publication (Wright et al., 2011). It will be important to determine whether the molecular mechanism described by Traver and colleagues also functions in mammalian development. This study very nicely confirms and illustrates the existence of specific, sequential windows of time at which defined combinatorial environmental cues ultimately lead to HSC specification. A similar signaling requirement that specifies cell fate choices—long after the signals themselves were active—was recently shown for BMP in the in vitro generation of blood cells from ESCs (Chiang and Wong, 2011). Molecular programs that propagate over time by either non-cell-autonomous relay signaling or cell-intrinsic deterministic mechanisms are not only highly interesting as models for molecular regulation. In combination with the required timed presence of many different cell types and their inductive signaling, they can also offer an explanation for the remaining difficulty in inducing HSCs from pluripotent cells (Cerdan and Bhatia, 2010). Hopefully, these novel insights will contribute to the comprehensive understanding of the required combinatorial timed signals (Ciau-Uitz et al., 2010) that will allow the efficient generation of unlimited, well-defined, and clinically applicable HSCs in vitro.

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Pluripotency without Max

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Myc/Max complexes are thought to be essential for maintaining pluripotency and self-renewal of embryonic stem cells (ESCs). In this issue of *Cell Stem Cell*, Hishida et al. (2011) provide genetic evidence that this requirement can be bypassed in well-defined culture conditions.

Due to its pervasive involvement in human tumorigenesis, the Myc oncoprotein and two of its cousins, N-Myc and L-Myc, have been under intense scrutiny for many years. More recently, endogenous c- and N-Myc proteins have been demonstrated to be individually or collectively essential for the self-renewal of embryonic and adult (e.g., hematopoietic) stem cells (Smith and Dalton, 2010). Importantly, deregulated expression of Myc, as is the hallmark of many human tumors, enhances the formation of induced pluripotent stem cells (iPSCs), suggesting that the oncogenic functions of Myc may be mechanistically related to its ability to confer self-renewal capacity to differentiated cells given the right genetic context (Takahashi and Yamanaka, 2006). As a result, the biochemical analysis of Myc function, particularly in ESCs, has begun to set paradigms for the study of Myc in human tumors.

Myc has an essential role in maintaining ESCs in a proliferative, self-renewing, and undifferentiated state. Together with a set of interacting proteins, Myc binds to a large set of promoters that are distinct from promoters bound by other factors involved in maintaining the pluripotency