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Retinoic Acid Regulates Hematopoietic Development from Human Pluripotent Stem Cells

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SUMMARY

The functions of retinoic acid (RA), a potent morphogen with crucial roles in embryogenesis including developmental hematopoiesis, have not been thoroughly investigated in the human setting. Using an in vitro model of human hematopoietic development, we evaluated the effects of RA signaling on the development of blood and on generated hematopoietic progenitors. Decreased RA signaling increases the generation of cells with a hematopoietic stem cell (HSC)-like phenotype, capable of differentiation into myeloid and lymphoid lineages, through two separate mechanisms: by increasing the commitment of pluripotent stem cells toward the hematopoietic lineage during the developmental process and by decreasing the differentiation of generated blood progenitors. Our results demonstrate that controlled low-level RA signaling is a requirement in human blood development, and we propose a new interpretation of RA as a regulatory factor, where appropriate control of RA signaling enables increased generation of hematopoietic progenitor cells from pluripotent stem cells in vitro.

INTRODUCTION

During embryonic development, retinoic acid (RA) acts as a morphogen, providing signals that instruct commitment of unspecified precursors toward separate cell fates, thereby helping to mediate tissue patterning and organogenesis (Duester, 2008; Kumar and Duester, 2011; Ross et al., 2000). As such, RA is also a potent teratogen capable of disturbing developmental processes, causing severe malformations of the fetus. RA is a signaling molecule derived from vitamin A (retinol), regulating cellular proliferation and differentiation, and is produced by cells that express the enzymes retinaldehyde dehydrogenase 1 (RALDH1), RALDH2, or RALDH3. A small molecule, diethylaminobenzaldehyde (DEAB), inhibits the activity of these enzymes and can be used to limit endogenous RA signaling (Moreb et al., 2012; Perz-Edwards et al., 2001; Russo and Hilton, 1988). When available, RA enters the nucleus to bind the retinoic acid receptor (RAR) family of nuclear receptors that, in turn, by forming heterodimeric complexes with the retinoid-X-receptor (RXR) family, localize to specific retinoic acid response elements (RAREs) in promoter regions of the genome to drive transcription of RA target genes. Modulation of RA in in vitro models of development provides a useful tool toward understanding commitment into tissues that depend either on high levels of RA, such as the developing ectodermal and endodermal derivatives (Murry and Keller, 2008) or on low levels of RA such as the posterior patterning of the lateral plate mesoderm (LPM) (Deimling and Drysdale, 2009). While several model organisms including *Xenopus* (*Xenopus laevis*), zebrafish (*Danio rerio*), and mouse (*Mus musculus*) have been used to study the effects of RA on embryonic development, similar studies have not been possible in the human setting for both technical and ethical reasons. In addition to its role in embryonic development, RA is also known to have strong effects on the hematopoietic lineage. However, the studies on the role of RA in regulating adult hematopoiesis have yielded opposing results. For example, RA signaling inhibition expands hematopoietic progenitors in both the human and murine setting (Chute et al., 2006; Muramoto et al., 2010), while other studies report increased maintenance and self-renewal upon adding RA to short- and long-term repopulating hematopoietic stem cells (HSCs) in the murine setting, mediated through activation of the RARγ receptor (Purton et al., 2000, 2006). These apparent contradictions likely reflect the increasingly complex nature of RA signaling that is emerging in the field, which is why a more contextual understanding might provide a better model to explain these previous findings. Indeed, work analyzing the effects of RA on different subpopulations of adult blood demonstrated that RA has a pleiotropic effect, enhancing the self-renewal of the HSC compartment while accelerating the differentiation of downstream progenitors (Purton et al., 1999). Several groups have created protocols that allow in vitro generation of hematopoietic cells from human pluripotent stem cells (Kardel and Eaves, 2012; Murry and Keller, 2008; Woods et al., 2011), with more

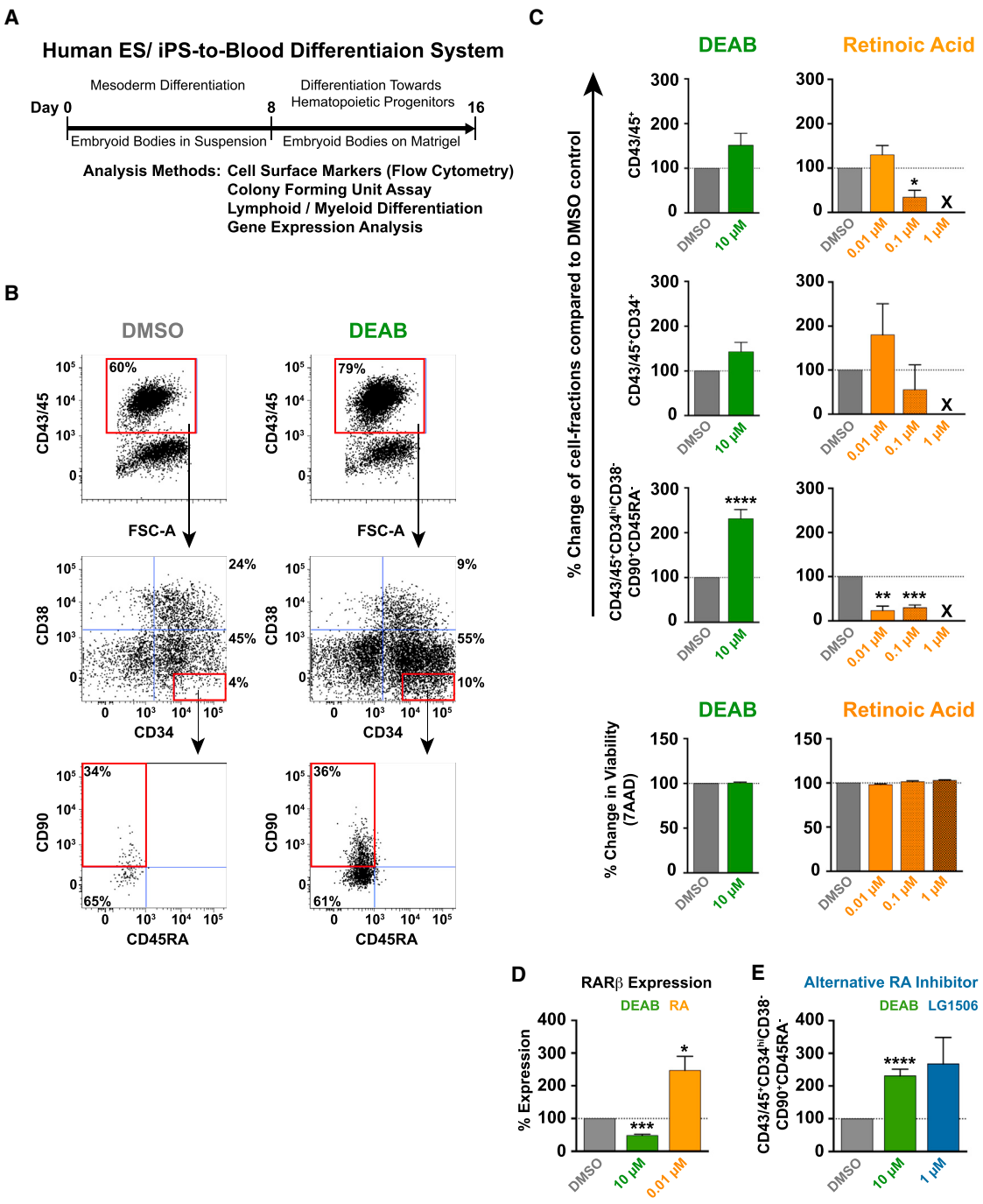

Figure 1. Retinoic Acid Signaling Inhibition Increases the Yield of Hematopoietic Progenitors with an HSC-like Phenotype from Human Pluripotent Stem Cells

(A) Schematic of pluripotent stem cell differentiation system used to model human hematopoietic development through mesoderm specification and blood commitment. RA inhibitors or RA was present continuously during the 16-day differentiation, except where otherwise stated.

(B) Representative FACS plots showing the hematopoietic population derived from pluripotent stem cells at day 16 of differentiation. FACS gates show blood ($CD45/43^+$), hematopoietic progenitors ($CD45/43^+CD34^+$), and HSC-like immature progenitors ($CD45/43^+CD34^{hi}CD38^{lo}CD90^+CD45RA^-$). Gates are based on FMO controls with more stringent $CD34^{hi}$ and $CD38^{lo}$ gating based on cord blood hematopoietic stem and progenitor cell standards. Doublet exclusion and dead cell exclusion were done before applying the gates.

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