

Lymphohematopoietic licence: Sterol C-14 reductase activity of lamin B receptor (Lbr) is essential for neutrophil differentiation

Bertie Wooster, PG Wodehouse's fictional character, proudly fancied himself a writer for having once contributed an article ('What the Well-Dressed Man Is Wearing') to *Milady's Boudoir*, his Aunt Dahlia's weekly magazine for women. My conceit of expertise in vertebrate lamin B receptor (Lbr) research is slightly less dubious. I co-authored two papers (Papavinasasundaram and Kasbekar 1994 and Prakash *et al.* 1999) that established that the C-terminal two-thirds of Lbr has sterol $\Delta^{14,15}$ reductase activity. An interloping article (Silve *et al.* 1998) reached pretty much the same conclusion (and to my chagrin, garnered the lion's share of the citations). So the recent demonstration by Peter Gaines and coworkers that the Lbr sterol reductase regulates differentiation of neutrophils (Subramanian *et al.* 2012) filled me with proprietary pride, especially since neutrophils are the most abundant white blood cells in circulation and present the critical first line of defence against infectious microbes.

Lbr is an integral protein of the vertebrate nuclear envelope inner membrane. Its N-terminal ~200 residues are hydrophilic, bind to B-type lamins, DNA and HP1-type chromatin proteins, and provide a substrate for p34^{cdc2}, a key mitotic protein kinase. The ~420 residue hydrophobic, membrane-spanning, C-terminal domain (CTD) with sterol reductase activity anchors the nucleoplasmic domain to the inner nuclear membrane. Mutations in human *LBR* cause Pelger-Huët anomaly (PHA), a benign dominant disorder characterized by hyposegmentation of the neutrophil nucleus (Hoffmann *et al.* 2002). A spontaneously aborted fetus with Greenberg/HEM dysplasia was homozygous for *LBR* mutations, and peripheral blood neutrophils from the fetus' mother displayed PHA (Waterham *et al.* 2003). Greenberg/HEM dysplasia and PHA reflect the pleiotropism of *LBR* mutations. In mouse, the *Lbr* gene is defined by the *ichthyosis* (*ic*) mutations (Shultz *et al.* 2003). Neutrophils from *ic/ic* mice display bilobed or ovoid nuclei typical of PHA. Additionally, *ic/ic* homozygotes exhibit sparse hair, decreased body size and occasionally hydrocephalus and syndactyly. It was of interest to understand the functional significance of the Lbr protein's sterol reductase activity, especially since KO mice for another locus, *Tm7sf2*, that encodes SR-1, a 418 residue protein with 58% identity with the Lbr CTD and possessing sterol C-14 reductase activity, do not display an observable phenotype (Bennati *et al.* 2006, 2008).

First, a quick flashback to another 1994 paper: Tsai *et al.* (1994) had shown that transduction of normal mouse bone marrow cells with a retroviral vector harbouring a dominant-negative retinoic acid receptor (RAR α 403) could reproducibly immortalize lymphohematopoietic progenitors as stem-cell-factor-dependent clonal lines, designated as EML cells for their ability to subsequently undergo erythroid, myeloid and lymphoid differentiation *in vitro*. A 3-day treatment of EML cells with stem cell factor, IL-3, and high concentrations of all-trans retinoic acid, and then washing and switching them into GM-CSF, induced their differentiation into promyelocytes, designated as EPRO cells (EML-derived promyelocytes), that can be maintained in GM-CSF. Treatment of EPRO cells with high concentrations of retinoic acid in the presence of GM-CSF induced them to terminally differentiate into mature neutrophils with characteristic nuclear lobulation and respiratory burst response phenotypes. Several years later, Gaines *et al.* (2008) generated EML- and EPRO-like cells from bone marrow of a C57BL/6J-*Lbr*^{*ic-J*}/*Lbr*^{*ic-J*} (*ic/ic*) mouse and a normal (+/*ic*) littermate, and found that neutrophils derived from EPRO-*ic/ic* cells exhibited nuclear hypolobulation identical to that seen in ichthyosis mice and displayed a deficient respiratory burst, whereas those from

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EPRO-+/ic, like their EPRO-+/+ counterparts, showed the multilobed or ringed nuclear morphology of normal neutrophils and displayed a normal respiratory burst. Fast-forwarding to the work of Subramanian *et al.* (2012), EPRO-+/ic and -ic/ic cells transduced with a FLAG-Lbr expression vector could be induced with retinoic acid to differentiate into neutrophils with normal nuclear lobulation, whereas very few ic/ic cells transduced with the empty vector showed any signs of lobulation. Surprisingly, a small but significant number of EPRO-ic/ic cells transduced with vectors expressing either the Lbr-71-626 or Lbr-145-626 mutant proteins, which lacked the N-terminal lamin B binding domain, or this plus the chromatin binding domains, respectively, also exhibited normal nuclear morphological maturation and improved respiratory bursts. These results suggested that nuclear lobulation is more critically dependent on the sterol reductase activity of Lbr CTD than on the Lbr nucleoplasmic domain. Significantly, overexpression of *Tm7sf2* did not rescue the growth and differentiation defects of EPRO-ic/ic cells. Moreover, when bone marrow of a *Tm7sf2* KO mouse and a genotypically normal littermate were transduced with the dominant-negative RAR α 403 construct, both resulting cell lines were able to form EPRO-like cells that upon induction with retinoic acid differentiated into morphologically mature neutrophils, consistent with the earlier results showing *Tm7sf2* KO mice do not have any observable phenotype. The requirement of the Lbr CTD for neutrophil differentiation from EPRO cells suggests it is required for normal neutrophil differentiation in bone marrow as well.

Gaines and coworkers are now all set to use their ic/ic cell line to study the effects of additional *Lbr* variants, including those that cause Greenberg/HEM dysplasia but not PHA in humans. I like to think that if they were to ask Jeeves, Bertie Wooster's 'gentleman's personal gentleman', he would suggest two additional papers as 'improving' reading: Prakash and Kasbekar (2002a), that identified a conserved tyrosine residue (homologous to Y445 in the *Neurospora* sterol 14-reductase) whose mutation we predicted will specifically excise Lbr sterol reductase activity, and Prakash and Kasbekar (2002b), that reported the failure of human SR-1 to complement sterol C-14 reductase mutants of *Neurospora* and yeast. Might Lbr CTD/*Tm7sf2* chimeras rescue the ic/ic phenotype?

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