Generation of Id1/GFP knock-in mice

To generate Id1/GFP knock-in construct, a genomic DNA fragment containing 2-kb of the 5' flanking and untranslated region of the Id1 gene was inserted through the artificial EcoRI and BamHI sites into a vector containing EGFP and floxed pGK-Neo, which was created by inserting a BamHI-NotI (blunted) fragment containing EGFP from the pEGFP-N1 vector (Clontech, Mountain View, CA) into BamHI and XbaI (blunted) sites of the ploxP vector[1]. A 4-kb SalI-NotI fragment containing the Id1 coding and 3' flanking sequences was then inserted downstream of the floxed pGK-Neo at the XhoI and NotI sites. This final construct was linearized by NotI digestion and used for eletroporation of 129X1/SvJ ES cells.

Two independent 129X1/SvJ ES cell lines harboring an appropriately targeted allele were identified by Southern blotting and PCR, and injected into the blastocysts of C57BL/6J mice. Chimeras were screened for germline transmission of the targeted allele. Positive progenies were crossed with EIIa-Cre transgenic mice on the C57BL/6 background to remove the neomycin-resistance gene in germ cells[2]. Mice carrying the knock-in allele and the EIIa-Cre transgene were identified by analyzing the tail DNA using PCR and Southern blotting and backcrossed with C57BL/6 mice. Mice, which have the pGK-neo cassette deleted from the knock-in allele and lack the EIIa-Cre transgene, were selected for subsequent backcrosses with C57BL/6 for three generations. The progenies were then crossed for at least two additional generations to B6-CD90.1 mice in order to obtain offspring that were homozygous at the CD90.1 locus. B6-$Id1^{+/GFP}$-CD90.1 mice were then interbred to obtain the $Id1^{GFP/GFP}$, $Id1^{+/GFP}$, and $Id1^{+/+}$ mice used in these studies. Id1/GFP genotyping was conducted by PCR analyses with the following primers: Id1-5b, CAACAGCATCTGGGAATCCTTGAC and Id1-R1, GCAGCGGCTGCGGCACTGCCACTG, which generates 1200-bp and 500-bp fragments representing knock-in and wild-type alleles, respectively. Knock-in allele genotyping was further verified by PCR with primers, Id1-5b and GFP-R (GCATGGCGGACTTGAAGAAGTC).

Reference List

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