Characterization and Analysis of MafB GENSAT BAC transgenic mice

GENSAT is a NIH-funded project that was initiated to generate BAC/EGFP transgenic lines with the intention to provide genetic tools that would facilitate the study of the central nervous system (CNS). We have take advantage of the availability of GENSAT transgenic mice to address whether any of the transgenic lines that have been generated would be appropriate to study renal development. The analysis here provides the kidney research community with basic information as to the utility of GENSAT transgenic strains in furthering the study of kidney development. As part of the GUDMAP consortium, we have tested several strains from GENSAT at a single appropriate time point (E15.5) and screened the mice for their ability to aid in the isolation of specific components from the developing kidney for gene expression profiling. Here we report the pattern of EGFP expression in the embryonic day 15.5 kidney of the *MafB*-EGFP strain. **Our analysis suggests that the *MafB-EGFP* transgenic mice may be useful in studying podocyte cell development.**

MafB Gene Notes

The protein encoded by this gene is a basic leucine zipper (bZIP) transcription factor that plays an important role in the regulation of lineage-specific hematopoiesis. The encoded nuclear protein represses ETS1-mediated transcription of erythroid-specific genes in myeloid cells. This gene contains no introns. Acts as a transcriptional activator or repressor. Involved in renal tubule survival (Moriguchi *et al*.).

Strain Information

Strain Name: STOCK Tg(Mafb-EGFP)79Gsat/Mmcd
Promoter: Mafb
Name: v-maf musculoaponeurotic fibrosarcoma oncogene family, protein B (avian)
Chromosome: 2
Alteration at locus: Transgenic Reporter: EGFP (Jelly Fish)
Name: Enhanced Green Fluorescent Protein
Alteration at locus: Transgenic

For further information and strain distribution please use the following
http://www.mmrrc.org/strains/11834/011834.html

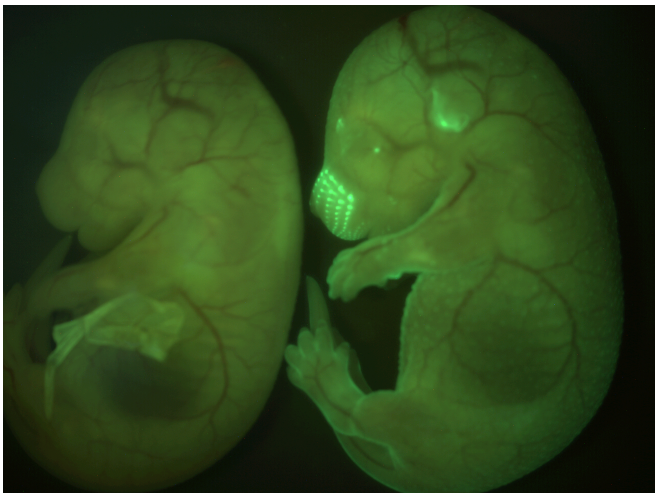

Figure 1. Analysis of *MafB*-EGFP expression in E15.5 embroys. Fluorescent microscopic image detailing expression of *MafB* at E15.5. The embryo on the left is a non-transgenic littermate, while the embryo on the right is a *MafB*-EGFP BAC transgenic. Note the particularly strong expression in the vibrassae.

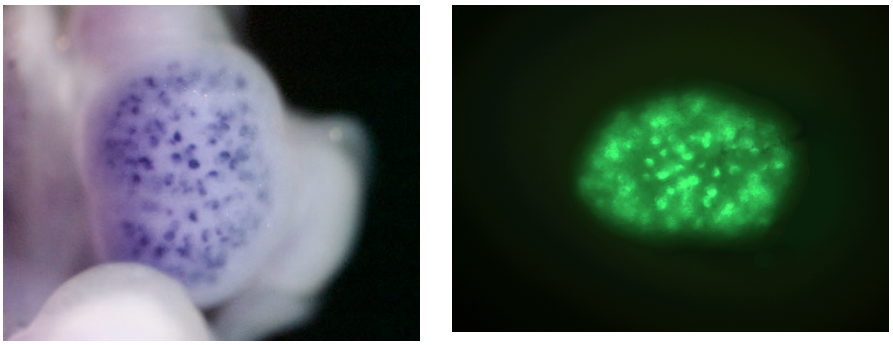

Figure 2. Expression pattern of *MafB*-EGFP in the kidney of E15.5 transgenic mice. The left side of the panel shows an *in situ* hybridization localization of *MafB* transcripts in the developing kidney at E15.5 (Image courtesy of Gudmap, McMahon Lab). The right side panel shows a fluorescent microscopic image from a *MafB*-EGFP kidney at E15.5. Note the punctate GFP expression, which marks the cells of the developing podocytes found in the S-shape body and glomerulus.

Characterization of MafB expression in the developing kidney cont.

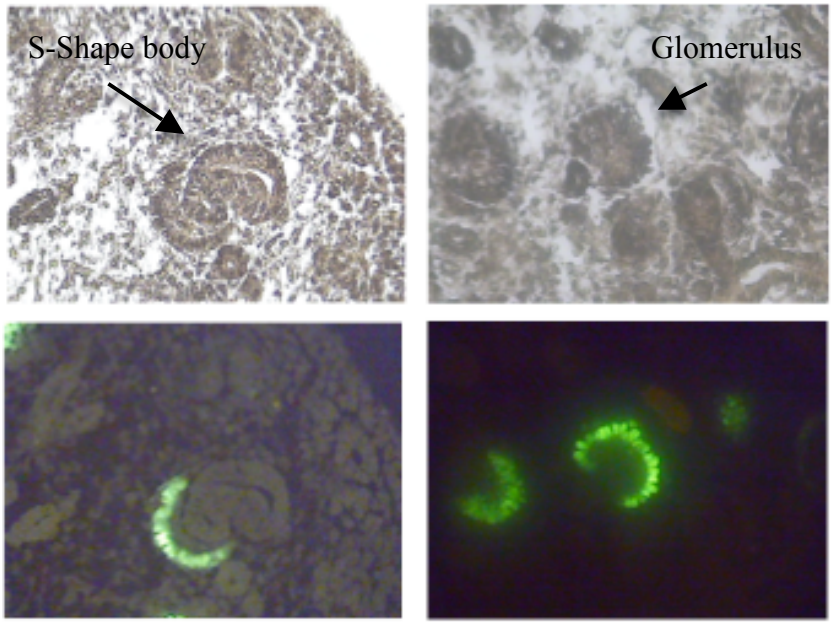

Figure 3. Expression pattern of *MafB*-EGFP in the kidneys of E15.5 transgenic mice. The top panels represent bright-field images while those on the bottom panels are fluorescent microscopic images. The images on the left show *MafB* expression in developing podocytes in the S-shape body, while those images on the right demonstrate expression of *MafB* in podocytes of the developing glomerulus.

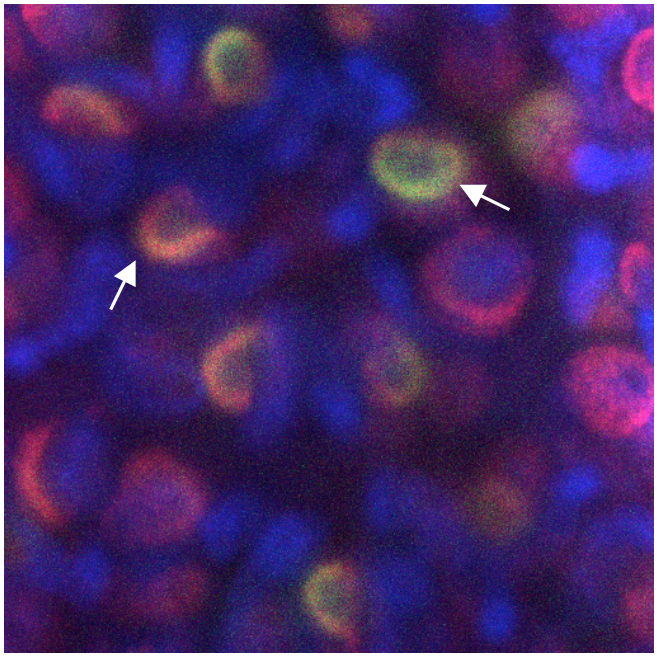

Figure 4. Confocal microscopic analysis of *MafB*-GFP expression in the developing kidney at E15.5. To further delineate and localize the expression pattern of *MafBb*-GFP in the kidney, we performed confocal analysis. This confocal image details the expression of *MafB*-GFP, which can be seen in the developing podocytes cells of the glomerulus (arrows). The tubules of the kidney were labeled with E-cadherin, and the mesenchyme and developing glomeruli labeled by WT-1. MafB (green), E-cadherin (blue), WT-1 (red).

Methods

Tissue processing for confocal microscopy

Kidneys were dissected in phosphate buffered saline (PBS). The kidneys or the organ explants were rocked for 1–2 h in 2% paraformaldehyde in PBS, washed twice with PBS, and then rocked for 1–2 h in 100% methanol. The tissues were washed twice with cold PBS containing 0.05% Tween-20 (PBT). Kidneys were bisected. Primary antibodies, diluted to 1:250 to 1:400, were added to the tissues in 400 μL of PBT containing 2% goat serum and incubated overnight with rocking. Tissues were washed with 5 exchanges of PBT over 8 h with rocking. The secondary antibodies, diluted to 1:400 in PBT containing 2% goat serum, were added and incubated overnight. The tissues were again washed with 5 exchanges of PBT over 8 h. The tissue was washed for 5–10 min and mounted in a depression slide in PBT before they were examined by confocal microscopy. The entire procedure was performed at 4 °C with pre- cooled reagents.

The following primary antibodies were utilized: anti-WT1 (c-19, Santa Cruz), anti-Uvomorulin (E-cadherin, Sigma). The secondary antibodies were Alexa 555-conjugated anti-rabbit and Alexa 633-conjugated anti-rat secondary antibodies (Molecular Probes).

Confocal imaging

The tissues were imaged with a Zeiss LSM510 equipped with an Argon (488 nm) and two HeNe lasers (543 nm and 633 nm). We used a multi-track configuration, refractive index correction, and automatic gain control. Approximately 2 μm thick optical sections were obtained every 5 μm to a depth of at least 80 μm. The sections began at the surface of the kidney and were on a plane tangential to it.

References

Gong S, Zheng C, Doughty ML, Losos K, Didkovsky N, Schambra UB, Nowak NJ, Joyner A, Leblanc G, Hatten ME, Heintz N. A gene expression atlas of the central nervous system based on bacterial artificial chromosomes Nature. 2003 Oct 30;425(6961):917-25.

GENSAT Project, Howard Hughes Medical Institute, The Rockefeller University, 1230 York Avenue, Box 260, New York 10021, USA."The Gene Expression Nervous System Atlas (GENSAT) Project, NINDS Contracts N01NS02331 & HHSN271200723701C to The Rockefeller University (New York, NY)."

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