Mdr1a/b-Bcrp Triple Targeted Mutation Mice

Targeted deletion of genes for the three drug transporters Mdr1a, Mdr1b and Bcrp provides a unique model for drug development, toxicity, CNS and oncology studies.

Applications of the Mdr1a/b-Bcrp Targeted Mutation Mouse Model

Taconic's Mdr1a/b-Bcrp Targeted Mutation Mice are homozygous for the targeted deletion of three genes: *Abcb1a*, *Abcb1b*, and *Abcg2*. These genes encode for three drug-extruding transporters. The closely related Mdr1a and Mdr1b murine transporter proteins are equivalent to the MDR1 protein in humans. Murine Bcrp is equivalent to human BCRP. The Mdr1a P-glycoprotein serves as the major P-glycoprotein of the blood-brain barrier. Disruption of Mdr1a and Mdr1b results in increased sensitivity to drugs and increased brain penetration of certain substances.[1] Bcrp is a member of the ATP-binding cassette (ABC) family of drug transporters. Bcrp knockout mice show compromised cellular excretion of certain substrates. They also display a unique protoporphyria.[2] All three of the disrupted genes are also implicated in multi-drug resistance in tumor cells, resulting from increased transport of chemotherapeutics from cells.[1,2]

Applications include:

- **Neurotoxicology**: study of the blood-brain barrier and neurotoxicity.
- **Drug transport**: test new approaches for promoting or minimizing access to the brain.
- **Oral bioavailability**: study the role of drug transporters in compound uptake.
- **Oncology**: research into drug resistant tumors.
- **Pharmacokinetics**: establish the contributions of various drug transporters to the pharmaco-kinetics of compounds of interest.
- **Teratology**: evaluate the barrier function of drug transporters in placental transport and susceptibility to chemically-induced birth defects.
- **Stem cell research**: evaluation of dye-excreting phenotype associated with stem cells in various tissues.

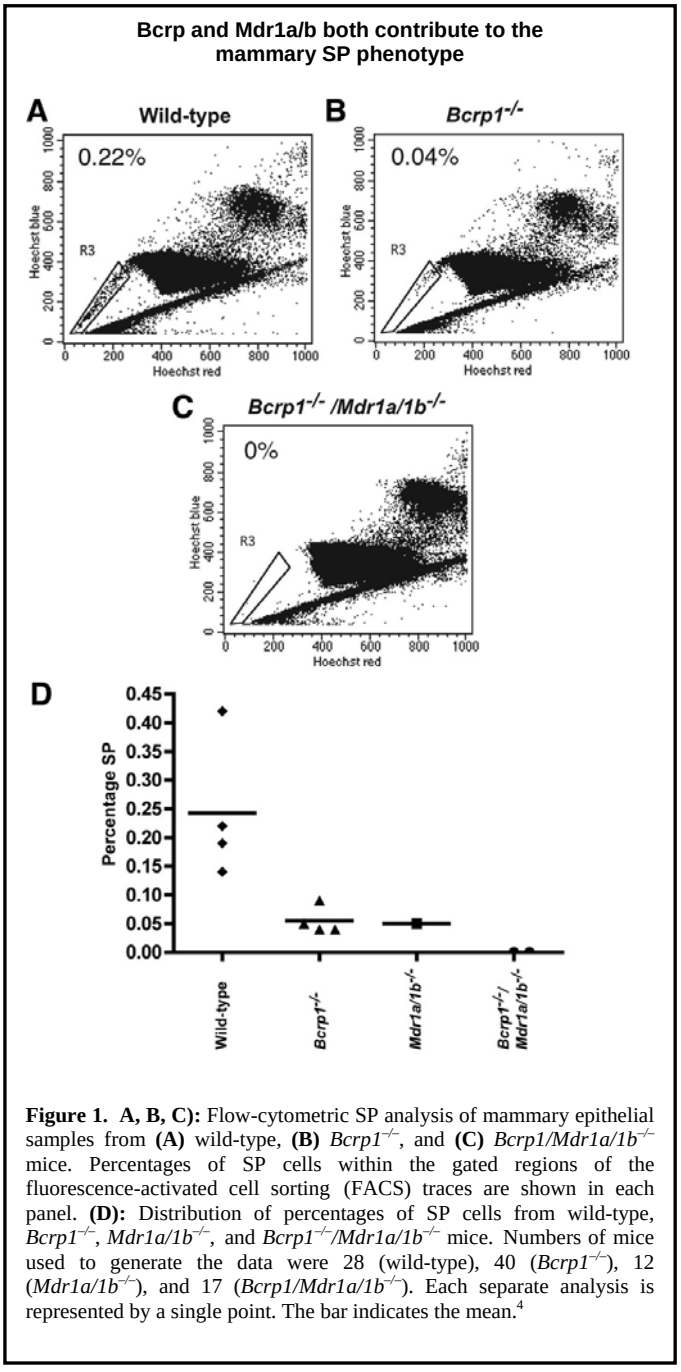

Figure 1. A, B, C): Flow-cytometric SP analysis of mammary epithelial samples from **(A)** wild-type, **(B)** *Bcrp1*$^{-/-}$, and **(C)** *Bcrp1/Mdr1a/1b*$^{-/-}$ mice. Percentages of SP cells within the gated regions of the fluorescence-activated cell sorting (FACS) traces are shown in each panel. **(D):** Distribution of percentages of SP cells from wild-type, *Bcrp1*$^{-/-}$, *Mdr1a/1b*$^{-/-}$, and *Bcrp1*$^{-/-}$*/Mdr1a/1b*$^{-/-}$ mice. Numbers of mice used to generate the data were 28 (wild-type), 40 (*Bcrp1*$^{-/-}$), 12 (*Mdr1a/1b*$^{-/-}$), and 17 (*Bcrp1/Mdr1a/1b*$^{-/-}$). Each separate analysis is represented by a single point. The bar indicates the mean.[4]

Features of Mdr1a/b-Bcrp Targeted Mutation Mice (model 003998)

- Homozygous disruption of three drug transporter genes.
- Mdr1a and Bcrp single knockout animals as well as Mdr1a/b double knockout animals on the same genetic background are available as controls.
- Animals are healthy and develop normally.

Scientific Profiles of the Mdr1a/b-Bcrp Targeted Mutation Mouse Models

Mdr1a/b targeted mutation mice display increased penetrance of drugs into the brain and decreased excretion of certain compounds. Both Mdr1a and Mdr1b are found in liver, kidney, lung, heart and spleen. Mdr1a is also found at the blood-brain and blood-testis barriers, and Mdr1b is expressed in the adrenal gland, ovaries and pregnant uterus. Study of the related Mdr1a and Mdr1a/b knockout animals revealed pharmacokinetic alterations. In Mdr1a and Mdr1a/b targeted mutation mice, digoxin concentrations in brain, testis and plasma were elevated compared to wild type controls. In addition, intestinal excretion of digoxin in both models was significantly reduced compared to wild type animals.[1]

Disruption of Mdr1a can lead to increased sensitivity to drugs. $Mdr1a^{-/-}$ mice exhibited a 100-fold increased sensitivity to the neurotoxic pesticide ivermectin compared to wild types. Knockout animals treated with a dilute solution of ivermectin to treat mite infestation died from toxicity, whereas wild type littermates showed no ill effects at the same concentration.[3]

Bcrp targeted mutation mice have compromised cellular excretion of certain substances. These mice are very sensitive to the normally non-toxic dietary component pheophorbide a. In normal mice, pheophorbide a uptake is limited, but Bcrp knockout animals accumulate the compound, resulting in severe and even lethal phototoxic lesions.[2]

Bcrp knockout mice display a unique protoporphyria. Protoporphyrin IX levels were elevated 10 fold in erythrocytes of Bcrp targeted mutation mice. Elevation of this phototoxic heme precursor was independent of diet.[2]

Bcrp-/- mice have increased oral availability and fetal accumulation of the chemotherapeutic topotecan. Homozygous knockout animals showed approximately 6-fold higher oral availability of topotecan as measured by plasma concentrations.[2]

Mdr1a/b-Bcrp targeted mutation mice were used to determine the relative contributions of the different p-glycoprotein drug transporters to the drug efflux phenotype of SP cells. Export of Hoechst 33342 dye identifies a subpopulation of cells in many tissues with the characteristics of stem cells (called side population or SP cells). Comparison of Mdr1a/b-Bcrp and Mdr1a/b mice demonstrated that Bcrp is the main drug transporter responsible for dye efflux in bone marrow cells, whereas Mdr1a/b and Bcrp all contributed to the phenotype in mammary gland cells.[4]

Origins of the Models

The Mdr1a/b-Bcrp Targeted Mutation Mouse Model was developed by Dr. Alfred Schinkel and his colleagues at the Netherlands Cancer Institute. The model was created through crossbreeding of the Mdr1a/b targeted mutation mouse and the Bcrp targeted mutation mouse in the Schinkel lab. The Mdr1a/b model was created through sequential targeting of the Abcb1a and Abcb1b genes in E14 ES cells. Resultant chimeras were backcrossed to FVB/N for seven (N7) generations. The Bcrp model was created by targeting the Abcg2 gene in E14 embryonic stem cells derived from 129P2/OlaHsd mice and injecting the targeted cells into FVB blastocysts. Resultant chimeras were backcrossed to FVB/N for seven generations. Taconic received stock of the triple targeted mutation line in April 2005. The colony is maintained through mating of animals homozygous for all three mutations.

Ready for Your Experiments

Taconic's Mdr1a/b-Bcrp Targeted Mutation Models are produced in Isolator Barrier Unit (IBU™) facilities. Mice are shipped in Taconic Transport Cages (TTC™) and come with an up-to-date health report documenting their Murine Pathogen Free (MPF™) health status. Barrier housing conditions are recommended for maintenance of Mdr1a/b-Bcrp Targeted Mutation Mice.

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References Cited:

1. Deemter, L., Smit, J.J.M., van der Valk, M.A., Voordouw, A.C., Spits, H., van Tellingen, O., Zijlmans, J.MJ.M., Fibbe, W.E., Borst, P. (1997) **Normal Viability and Altered Pharmacokinetics in Mice Lacking Mdr1-type (Drug-transporting) P-glycoproteins**. *Proceedings of the National Academy of Sciences USA*, Vol. 94, pp. 4028-4033.
2. Jonker, J.W., Buitelaar, M., Wganaar, E., van der Valk, M.A., Scheffer, G.L., Scheper, R.J., Plösch, T., Kuipers, F., Oude Elferink, R.P.J., Rosing, H., Beijnen, J.H., Schinkel, A.H. (2002) **The Breast Cancer Resistance Protein Protects Against a Major Chlorophyll-derived Dietary Phototoxin and Protoporphyria**. *Proceedings of the National Academy of Sciences USA*, Vol. 99, pp. 15649-15654.
3. Schinkel, A.H., Smit, J.J.M., van Tellingen, O., Beijnen, J.H., Wagenaar, E., van Deemter, L., Mol, C.A.A.M., van der Valk, M.A., Robanus-Maandag, E.C., te Riele, H.P.J., Berns, A.J.M. Borst, P. (1994) **Disruption of the Mouse Mdr1a P-Glycoprotein Gene Leads to a Deficiency in the Blood-Brain Barrier and to Increased Sensitivity to Drugs**. *Cell*, Vol. 77, pp. 491-502.
4. Jonker, J.W., Freeman, J., Bolscher, E., Musters, S., Alvi, A., Titley, I., Schinkel, A.H., Dale, T.C. (2005) **Contribution of the ABC Transporters Bcrp1 and Mdr1a/1b to the Side Population Phenotype in Mammary Gland and Bone Marrow of Mice**. *Stem Cells*, Vol. 23, pp. 1059-1065.

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For more information or to place an order contact:

TACONIC
One Hudson City Centre
Hudson, NY 12534
Toll Free: 1-888-TACONIC
Phone: 518-537-6208
Fax: 518-537-7287
e-mail: custserv@taconic.com
Internet: http://www.taconic.com

in Europe: Taconic Europe
Bomholtvej 10 P.O. Box 39
DK 8680 Ry DENMARK
Phone: +45 70 23 04 05
Fax: +45 86 84 16 99
e-mail: TaconicEurope@taconic.com
Internet: http://www.taconic.com

in Japan: Immuno-Biological Laboratories, Co., Ltd.
5-1 Aramachi, Takasaki-Shi
Gunma 370-0831 JAPAN
Phone: +81 273-10-8040
Fax: +81 273-10-8045
e-mail: do-ibl@ibl-japan.co.jp
Internet: http://www.ibl-japan.co.jp

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Related Mouse Models from Taconic

Taconic provides a number of mouse models relevant to immunology. Call or fax for information about these additional models:

- **Bcrp Targeted Mutation Mouse (model 002767)** – carrying a disrupted *Abcg2* gene. Associated with multi-drug resistance. Useful for studies of drug uptake and cellular transport.
- **HRN Mouse (models 007293 and 007353)** – carries a liver-specific deletion of the *Por* gene, resulting in a mouse that lacks hepatic cytochrome P450 activity.
- **Mdr1a Targeted Mutation Mouse (model MDR1A)** – carrying a disrupted *Abcb1a* gene, a multi-drug resistance-associated transport protein, conferring a deficiency in the blood-brain barrier; useful in neurotoxicology and in studies of drug design, cellular transport and testing
- **Mdr1a/b Targeted Mutation Mouse (model 001487)** – carrying disruptions of two genes, *Abcb1a* and *Abcb1b* and lacking cellular transport mechanisms by their two multi-drug resistance-associated protein products, conferring a deficiency in the blood-brain barrier; useful in neurotoxicology and in studies of drug design, cellular transport and testing.
- **Oct1/2 Targeted Mutation Mouse (model 006622)**– carrying a disruption of the *Slc22a1* and *Slc22a2* genes, which encode the organic cation transporters 1and 2.

Taconic Transgenic Models
Publication Reference List
Mdr1a/b-Bcrp Targeted Mutation Mice

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