Tissue Specificity for Mutation Parallels Tissue Specificity for Cancer

Johan G. de Boer • Barry W. Glickman
Centre for Environmental Health, University of Victoria, Victoria, BC Canada
Measure mutations in any tissue with the Big Blue® transgenic rodent mutation assay, which carries the E. coli lacI transgene in a retrievable chromosomally integrated shuttle vector, it is easy to study mutations in male and female mice or rats of any age under selected conditions in vitro. Multitab studies indicate that mutations are generally induced in the same tissue that tumors are produced but not in monogender tissues. This makes the transgenic model for the study of genotoxicity even more valuable, since mutations can be studied in virtually any tissue.

The hallmark of occupational- and lifestyle-related cancer is tissue specificity. Human exposures to carcinogens result in particular types of cancer at predictable sites; tobacco smoke, arsenic, and ionizing radiation all produce their own unique kinds of cancers in preferred tissues. Tissue specificity is also a feature of animal model systems. For example, when male mice are exposed to the flame retardant tris(2,3-dibromopropyl)phosphate (TDBP), they contract kidney tumors, and the alkylating agent dimethylamine predominately produces liver, kidney, and lung tumors. Sex can also have an impact on tissue targeting. In the case of TDBP, liver tumors predominate in female mice, while kidney tumors are seen in male mice.

This kind of information is crucial for identifying suspected carcinogens. Hence, we ask a critical question: Does mutation follow the same pattern as the targeting of tumors? In other words, are mutations being induced in tissues where tumors are recovered and not in others? Big Blue® transgenic rodents with recoverable mutation target genes make use of the well-characterized bacterial lacI gene as a mutational target gene. The lacI gene, part of the λLIZ shuttle vector, is present in approximately 40 copies per chromosome in the mouse and rat. Mutations are efficiently assessed through the recovery of the λ genome (Figure 1). High molecular weight genomic DNA is isolated from any selected Big Blue transgenic rodent tissue, which can be facilitated with the RecoverEase® DNA isolation kit, and the λ genomes are packaged into phage particles by Transpack® lambda packaging extract. The λ particles are then plated on a specially constructed E. coli host in the presence of the chromogenic compound X-gal. Phages carrying the
mutated *lacI* gene produce blue plaques, whereas the wild-type gene yields colorless plaques. The ratio of blue to clear plaques indicates the mutant frequency, which in most tissues varies between 2 to 6 x 10⁻⁴, although it is often lower in the rat than the mouse. Subsequent sequencing of the mutant *lacI* genes reveals the specific nature of the DNA change at the molecular level; when a sufficient number of mutants have been sequenced, a mutational spectrum results. Mutational spectra are extremely useful in assessing the mechanism of mutation. DNA sequencing also permits the mutant frequency to be adjusted by allowing a correction for clonal expansion in which an individual mutation is recovered multiple times because of cell division.

### Tissue Specific Effects

Researchers who have designed studies to assess mutations in a range of tissues after carcinogen treatment have addressed whether mutations are induced selectively in those tissues targeted for tumorigenesis. We reviewed some of the available data to illustrate how the Big Blue transgenic rodent mutagenesis assay system is used in tissue-specific studies (Table 1).

**TDBP**

The flame retardant tris(2,3-dibromopropyl)phosphate (TDBP) is an established kidney-specific carcinogen in male and female rats and male mice; in female mice, it targets the liver. In a TDBP study, the *lacI* mutation frequency was determined in the kidney, liver, and stomach of male Big Blue mice after treatment with 2000 ppm of TDBP. The mutant frequency increased marginally in the kidney and liver (approximately 50%) but not the stomach. Sequencing the mutants revealed a unique difference in mutational spectrum: The spectrum from the liver was indistinguishable from that of untreated animals, while that from the kidney included unique deletions of single GC base pairs. Thus, in this case, TDBP-specific mutations were induced only in the tissue where tumors were recovered. Hence, DNA sequencing can improve the assay sensitivity in situations where the induced mutant frequency is low.

**DMN**

Tissue specificity was also observed with dimethylazinrosamine (DMN), which has been well studied in the liver. DMN produces tumors in various organs including the liver, kidney, and lung. Suzuki et al. observed mutation induction in each of these tissues (6.2, 2.4, and 2.1 fold, respectively). In contrast, they observed no statistically significant mutation induction in nontarget tissues such as the urinary bladder (1.7 x 10⁻⁴ versus 1.4 x 10⁻⁴ in controls), testis (0.39 x 10⁻⁴ versus 0.48 x 10⁻⁴ in controls), and bone marrow (1.7 x 10⁻⁴ versus 1.3 x 10⁻⁴ in controls).

**MelIQ**

MelIQ (2-amino-3,4-dimethylimidazo[4,5-f]quinoline) is a potent heterocyclic amine found in the human diet. MelIQ induces tumors in several rat tissues including colon, skin, mammary gland, oral cavity; in the mouse it forms tumors of the forestomach and liver. Several publications have shown that MelIQ induced mutations in Big Blue transgenic rodents. Induced mutations have been observed in each target tissue except the heart, which is not a target for tumorigenesis. Ushijima et al. determined the DNA sequence alterations in mutants recovered from the liver (approximately a 4.5-fold increase in mutant frequency) and bone marrow (approximately a 5-fold increase). A significant tissue-specific difference was seen in the mutant fractions that were GC to TA transversions: 37% in the liver versus only 14% in bone marrow. Consequently, both sequencing DNA and determining mutant frequency are important for more accurately assessing mutation induction and mechanism.

**PhIP**

PhIP (2-amino-1-methyl-6-phenylimidazo[4,5-h]pyridine) is a colon carcinogen in the male rat but does not cause tumors in the liver. It induced a 20-fold increase in colon mutations after 400 ppm PhIP was added to the diet. We recently determined that no mutations above background are induced in the liver of these animals (de Boer, et al., unpublished). DNA adducts are found in the colon but not the liver. PhIP is metabolically activated in the liver by cytochrome P450, but additional metabolic processing takes place in colon cells.

**Ethyleneoxide and Benzene**

Sisk et al. studied mutation induction in *lacI* transgenic mice after the mice inhaled 200 ppm of ethylene oxide. The mutant frequency increased significantly in the lung (9.1 x 10⁻⁴ versus 6.2 x 10⁻⁴ in the controls) but not the spleen, bone marrow, and male germ cells. The lung is indeed a major target organ for tumor formation in the mouse. In another mouse inhalation study with benzene, which induces lung tumors, leukemia, and lymphomas, mutations significantly increased in the lung and spleen (a 1.7- and 1.5-fold increase, respectively) but not in the liver tissue. Benzene metabolism takes place partially in the liver, while final oxidation steps can take place in the bone marrow.

### Conclusions

The Big Blue transgenic rodent assay system accurately predicts mutations in specific tissues. In a number of
cases, mutations are recovered in tissues that are not targets for carcinogenesis. While this eases the screening for mutagenic compounds, it becomes more difficult to determine mutagenic mechanisms and how they relate to carcinogenesis. For example, we recently determined that mutation induction in the kidney after TDBP treatment is highest in the cortex layer and less in the outer and inner medulla (de Boer, et al., in preparation). Tumors, however, do not arise in the cortex but in the outer medulla layer. This and other findings indicate that cell proliferation is equally important for tissue-specific tumor formation.

REFERENCES

* U.S. Patent No. 5,347,075 and 5,389,155; European Patent No. 029921.
** U.S. Patent No. 5,188,957.

| Big Blue® Transgenic Mice | STRAIN | Big Blue Hemizygous | female | male | #720010
| | CS7BL/6 inbred | 40 copies of JLLZ per cell | | | #720011
| | Big Blue Hemoxygous | 80 copies of JLLZ per cell | | | #029085
| | B6C3F1 F1 hybrid | Hemizygous | male | #720020
| | TSG53/Big Blue | 40 copies of JLLZ per cell | | | #720021
| | Fischer 344 inbred | Male | Inquire | Male | Inquire
| | Big Blue® Transgenic Rats | | | | Inquire
| | Big Blue® Transgenic Cell Cultures | STRAIN | CaPO4 transfected | polyiod cell | #720600
| | Rat 2 Embryonic Fibroblasts | 50-70 copies of JLLZ per polyiod cell | | | #720610
| | Mouse Embryonic Fibroblasts | Derived from CS7BL/6 transgenic mice | | | #720071
| | Big Blue® Assay Materials and Reagents | CONTENTS | Big Blue® Assay Trays | 20 trays | 20 sterl  Big Blue assay trays | #400041
| | | 100 trays | 100 sterl  Big Blue assay trays | #400040
| | Big Blue® Media | CONTENTS | Big Blue® bottom agar formulation | #720030
| | 1 kg | Big Blue® top agar formulation | #720023
| | Transpack Packaging Extract | 50 packaging reactions | #200023
| | 100 packaging reactions | #200021
| | 400 packaging reactions | #200020
| | RecoverEase DNA Isolation Kit | 15 reactions from 100 mg of tissue | #720203
| | 30 reactions from 100 mg of tissue | #720202

Figure 1
The Big Blue® Transgenic Mice

1. Treat Big Blue® Rodent®
2. Prepare and package genomic DNA from tissue of interest with Transpack lambda packaging extract**
3. Infect G1250 off host and grow under selective and nonselective conditions

![Diagram of assay process]

The first two steps (animal treatment and packaging genomic DNA) are performed as in the lacI plaque color-screening assay. Packaged phage are then used to infect G1250 E. coli host strain and allowed to grow at 24°C, which selects for lambda cll mutant plaques. Determination of the total number of plaque screened is accomplished by titening at 37°C.

λ Select-cll® Mutation Assay Kit

- Positive selection resulting in more cost-effective and less labor-intensive assay
- One bacterial strain that reduces variability between selection and titer plates
- Small target gene for easy DNA sequence characterization
- Second genetic endpoint for parallel studies with the lacI plaque color-screening assay.

λ Select-cll® Mutation Assay Kit

- Positive selection resulting in more cost-effective and less labor-intensive assay
- One bacterial strain that reduces variability between selection and titer plates
- Small target gene for easy DNA sequence characterization
- Second genetic endpoint for parallel studies with the lacI plaque color-screening assay.

λ Select-cll® Mutation Assay Kit

- Positive selection resulting in more cost-effective and less labor-intensive assay
- One bacterial strain that reduces variability between selection and titer plates
- Small target gene for easy DNA sequence characterization
- Second genetic endpoint for parallel studies with the lacI plaque color-screening assay.

λ Select-cll® Mutation Assay Kit

- Positive selection resulting in more cost-effective and less labor-intensive assay
- One bacterial strain that reduces variability between selection and titer plates
- Small target gene for easy DNA sequence characterization
- Second genetic endpoint for parallel studies with the lacI plaque color-screening assay.