Available online at www.sciencedirect.com
SCIENCE DIRECT®
Mutation Research 578 (2005) 117–123
Fundamental and Molecular Mechanisms of Mutagenesis
www.elsevier.com/locate/molmut
Community address: www.elsevier.com/locate/mutres

A *lacZ* transgenic mouse assay for the detection of mutations in follicular granulosa cells

Carole L. Yauk [a], John D. Gingerich [a], Lynda Soper [a], Avril MacMahon [a], Warren G. Foster [b], George R. Douglas [a,*]

[a] *Environmental and Occupational Toxicology Division, Health Canada, Ottawa, Ont., Canada K1A 0L2*
[b] *Department of Obstetrics and Gynecology, McMaster Medical Centre, Hamilton, Ont., Canada L8N 3Z5*

Received 5 November 2004; received in revised form 31 March 2005; accepted 3 April 2005
Available online 21 July 2005

Abstract

There is ongoing concern that an assay for germ cell effects in female animals is not available. While transgenic mutation detection systems provide unprecedented access to numerous rodent tissues, studies on the induction of gene mutations in oocytes are still not possible because sufficient numbers of cells cannot be harvested. However, following stimulation of an ovarian follicle, the granulosa cells contained therein divide rapidly, increasing substantially in numbers. Since these granulosa cells share the same environment as the ovum, they may serve as suitable surrogates for the study of exposure of female germ cells to mutagens. Female *lacZ* transgenic mice (Muta^{TM}Mouse) were treated by intraperitoneal injection of *N*-ethylnitrosourea (ENU) and subsequently with pregnant mare serum gonadotropin (PMSG, 5 IU/animal, i.p.) to induce follicular growth. Animals were sacrificed 48 h after the administration of PMSG and granulosa cells and bone marrow were harvested. A comparable dose-related increase in the mutant frequency (MF) of both granulosa and bone marrow cells was observed. The highest dose caused a decrease in the MF of granulosa cells, but not in the bone marrow, suggesting possible greater susceptibility of granulosa cells to ENU toxicity. Doubling dose estimates for bone marrow and granulosa cells were lower than those derived from the literature on oocyte mutation frequency using the Russell specific locus assay, suggesting that both cell types are more sensitive to ENU-induced mutation than oocytes. The results indicate that transgene mutations in granulosa cells may provide a sensitive pre-screening tool for potential genotoxic germ cell effects of exposed oocytes.
Published by Elsevier B.V.

Keywords: *LacZ*; Oocytes; Mutagen; Mutation; Germline

1. Introduction

Most germ cell mutation research is performed in mice because of their small size, short generation time and the compendium of information available on the

* Corresponding author. Present address: Environmental Health Centre, Health Canada, Tunney's Pasture, P.L. 0803A, Ottawa, Ont., Canada K1A 0L2.
E-mail address: george_douglas@hc-sc.gc.ca (G.R. Douglas).

0027-5107/$ – see front matter. Published by Elsevier B.V.
doi:10.1016/j.mrfmmm.2005.04.011

mouse genome. Traditional approaches used to measure induction and transmission of germline mutation in vivo is time-consuming and costly (e.g., [1–4]). The majority of these assays rely on breeding and assaying to the F1 generation, and require large numbers of animals because germinal mutations are extremely rare.

Transgenic mutation-detection systems provide a cost-effective alternative to traditional assays by allowing the measurement of mutations arising, in principle, in any tissue within an animal [5,6]. In the traditional mutation-detection systems, transgenes integrated into the mouse genome are mutated in vivo as a result of exposure of the animal to a mutagen. The transgene is recovered, transferred to bacteria where the mutation detection assay is performed. Germline mutation evaluation has been undertaken by looking directly at gametes in males. For example, the technology has been applied to measure mutations induced by various genotoxins in male germ cells of transgenic mice to demonstrate the utility of these models for the study of spermatogonial and post-spermatogonial germ cell mutations [7–9]. Furthermore, Barnett et al. [10] showed that mutations induced in transgenes in male germ cells were transmitted to the next generation, and correlated with induced mutation at endogenous loci, thereby providing biological validation of the utility of this approach in germ cell studies.

The study of female germline mutation is severely limited by the number of oocytes available for evaluation. In females, meiosis is initiated during embryogenesis and arrested in the late diplotene (dictyate stage; 21 days post-coitus) of meiotic prophase (post crossing-over and recombination) until stimulated to resume meiosis at ovulation [11]. The number of oocytes in mammalian females is fixed at birth and decreases significantly with age as a result of follicular atresia; the lifetime total number of oocytes available is limited to several thousand, although recent evidence suggests that new oocyte-containing follicles continue to develop in the ovaries of adult mice [12]. Analysis of germ cell effects in females is also complicated by the extended period of time over which oogenesis occurs. Accordingly, there is ongoing concern that an adequate assay for germ cell effects in females is not available [10,13,14]. Since transgenic mutation detection systems require the isolation of sufficient DNA to detect rare mutation events, they are not applicable to direct measurement of mutation in oocytes sampled in vivo because of the low number of cells that can be recovered.

In this study, we investigated the utility of the *lacZ* Muta^{TM}Mouse model for the study of mutation in the granulosa cells surrounding the ovum in females exposed to ethylnitrosourea (ENU). We characterized mutation events occurring in bone marrow as a positive control as it has a high rate of proliferation and undergoes increased *lacZ* mutation as a result of exposure to ENU [8]. ENU is a well-characterized, direct acting-alkylating agent that is known to result in induced *lacZ* mutations in exposed spermatogonial and post-spermatogonial germ cells in the Muta^{TM}Mouse model [8]. Furthermore, ENU is known to induce germline mutation in females detected in offspring using traditional approaches [3,15]. Granulosa cells are the predominant cell type that make up the follicle and surround the egg. These cells divide rapidly following follicular stimulation and substantially increase in numbers. We hypothesized that the granulosa cells surrounding the oocyte which share the same environment as the oocyte may be suitable surrogates for the determination and study of mutagenic exposure of the ovarian environment.

2. Materials and methods

2.1. Transgenic animals and treatment

The transgenic mouse line 40.6 (Muta^{TM}Mouse; Corning Hazleton, Vienna, VA, USA) has previously been described [16]. Animals were bred locally and maintained under conditions approved by the Health Canada Animal Care Committee. Mice were maintained in a 12-h light:12-h dark regime and fed Ralston Purina Rodent Chow (Hazleton, PA, USA) and water ad libitum.

2.2. Treatment of animals

Five animals were housed per cage in Isotec plastic film isolators for exposures. Females aged 25 weeks were given single i.p. injections of 80 mg/kg ENU (Sigma–Aldrich, Oakville, Ont., Canada) dissolved in M/15 phosphate buffer, pH 6.0, alongside solvent controls. A single i.p. injection of pregnant mare's serum gonadotropin (PMSG; Sigma–Aldrich) was delivered