Available online at www.sciencedirect.com

SCIENCE DIRECT

Genomics 86 (2005) 271 – 279

GENOMICS

www.elsevier.com/locate/ygeno

Fine mapping of radiation susceptibility and gene expression analysis of LEC congenic rat lines

Atsushi B. Tsuji[a], Aya Sugyo[a], Toshiaki Ogiu[b], Masashi Sagara[a], Tomo Kimura[a], Atsuko Ishikawa[a], Hitomi Sudo[a], Marika Ohtsuki[a], Hiroyuki Aburatani[c], Takashi Imai[a], Yoshi-nobu Harada[a,*]

[a]*RadGenomics Project, Frontier Research Center, National Institute of Radiological Sciences, 4-9-1 Anagawa, Inage-ku, Chiba 263-8555, Japan*
[b]*Low-Dose Radiation Effects Project, Research Center for Radiation Safety, National Institute of Radiological Sciences, 4-9-1 Anagawa, Inage-ku, Chiba 263-8555, Japan*
[c]*Research Center for Advanced Science and Technology, The University of Tokyo, 4-6-1 Komaba, Meguro-ku, Tokyo 153-8904, Japan*

Received 28 September 2004; accepted 25 May 2005
Available online 22 June 2005

Abstract

LEC rats constitute an animal model of high susceptibility to X-rays. We developed congenic LEC rat lines (recipient strain, Fischer 344 (F344)) and performed genome-wide genotyping to identify radiation susceptibility genes. We mapped seven positional candidate genes, *Bmp10, Gpr73, Gp9, Cnbp, Copg, Rab7,* and *Rpn1*, to an ~1.2-Mb region located between loci *D4Got85* and *D4Got148* on chromosome 4. None of the seven genes has been reported to be associated with radiation susceptibility. Comparison of the coding sequences for these seven genes in F344 and LEC rats showed no changes in deduced amino acid sequences. We determined gene expression differences in *Gp73, Gp9,* and *Cnbp* as well as strain-specific variations in upstream sequences of these genes. Our results suggest that radiation susceptibility in the LEC rat is primarily attributable to one of the genes within this ~1.2-Mb region; however, expression analysis gave no clear indication as to which gene is responsible.

Keywords: X-rays; Whole-body irradiation; Radiation, ionizing; Animals, congenic; Rats, inbred LEC

Radiotherapy is widely and successfully applied to treat many malignant tumor types. In some patients, however, radiotherapy causes unacceptable levels of damage to normal tissues, including marked atrophy, severe edema, severe fibrosis, and dysfunction [1–3]. Variations in X-ray susceptibility may be attributable to a variety of genetic and environmental factors, including age, lifestyle, nutritional status, medication, and morbidity due to other diseases. For example, the genes responsible for ataxia telangiectasia and Nijmegen breakage syndrome are involved in radiation susceptibility [1,3]. However, the genetic factors responsible for susceptibility in nonsyndromic patients remain elusive. Although several genes related to DNA repair, cell cycle regulation, cell growth, cell death, carcinogenesis, and tumor suppression might be suspected to have a role in radiation susceptibility, at present there is no clear evidence for a relationship between such genes and radiation susceptibility in cancer patients. Consequently, a comprehensive survey of genes related to radiation susceptibility would help elucidate the molecular mechanisms underlying the physiological responses to X-ray exposure. The understanding of radiation-induced responses will lead to the development of a genetic-based assay to predict the radiation susceptibility of normal tissues in individual patients. Such an assay would

Abbreviations: *Atp7b*, ATPase, Cu^{2+} transporting, β polypeptide; *Bmp10*, bone morphogenetic protein 10; *Gpr73*, G-protein-coupled receptor; *Gp9*, glycoprotein IX; *Cnbp*, cellular nucleic acid binding protein; *Copg*, coatomer protein complex subunit γ; *Rab7*, Ras-related protein Rab-7; *Rpn1*, ribophorin I; *Actb*, β-actin; *Cebpa*, CCAAT/enhancer binding protein, α.

* Corresponding author. Fax: +81 43 206 4138.
E-mail address: y_harada@nirs.go.jp (Y. Harada).

0888-7543/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.ygeno.2005.05.007

improve the therapeutic ratio for curative radiotherapy and generate new protocols for personalized radiotherapy [1–3].

The LEC rat is a widely used animal model for fulminating hepatic disorder and Wilson disease in humans [4,5]. In addition, it is a spontaneous animal model of high susceptibility to X-rays [6–9]. Radiation susceptibility in the LEC rat is characterized by high mortality due to gastrointestinal [6] and hematopoietic syndromes [7,9] and impaired DNA repair [8] after X-ray exposure. Fibroblasts derived from the lung and tail of LEC rats also are highly susceptible to radiation [8]. It has been reported that each radiation-susceptible phenotype is controlled by either a single autosomal recessive gene [8] or multiple genes [9,10]. However, previous reports have failed to establish a link between radiation susceptibility and several potential candidate genes, such as the human Wilson disease-related *Atp7b*^{1} [9] and the DNA repair-associated *Rad52* gene [10]. These reports suggest that one or more novel genes might be causative for radiation susceptibility in the LEC rat. To identify this gene(s), we developed congenic lines using a phenotype-driven breeding protocol. Using these congenic lines and their progeny, we then localized the radiation susceptibility gene(s) to rat chromosome 4 in the region from *D4Got85* to *D4Got148*.

Results and discussion

Congenic lines and fine mapping of the radiation susceptibility gene

Results from our previous study [9] and from other reports [8,10] led us to the working hypothesis that the high susceptibility to X-rays in the LEC rat is associated with a single major gene and several minor genes. To identify the susceptibility gene(s), we developed congenic lines using an established backcrossing technique [11], whereby LEC rats were continuously backcrossed with Fischer 344 (F344) rats. Each backcross generation was then assessed for radiation susceptibility (Supplementary Fig. 1). At each backcross generation, approximately 50% of offspring yielded test-cross progeny with high susceptibility to radiation, thus supporting our working hypothesis of a single, autosomal recessive locus controlling this trait.

We performed a rough genome-wide genotyping analysis at the 7th backcross generation because congenic lines theoretically have <1% of the donor genome by the 7th generation [11] (Supplementary Fig. 1). Four congenic lines (A–D) at the 7th backcross generation that were highly susceptible to X-rays (as assessed indirectly through their progeny) were subjected to genome-wide genotyping analysis using 30 microsatellite markers. Three of four lines had an LEC allele at the *D4Rat36* locus on chromosome 4, and two had LEC alleles at loci *D19Rat12* and *D19Rat24* on chromosome 19 (Fig. 1). No LEC alleles were identified at other loci in these four lines. We genotyped 8 additional backcross offspring using 14 markers within the region from *D4Rat62* to *D4Rat19* and 11 markers within the region from *D19Rat16* to *D19Rat24*. This more refined analysis showed that all four lines had an LEC-derived chromosomal segment between *D4Got87* and *D4Got83* on chromosome 4, and only two of these lines had LEC alleles on chromosome 19 (Fig. 1), suggesting that at least one susceptibility gene is located between *D4Got87* and *D4Got83* on chromosome 4. At the 8th generation, 1

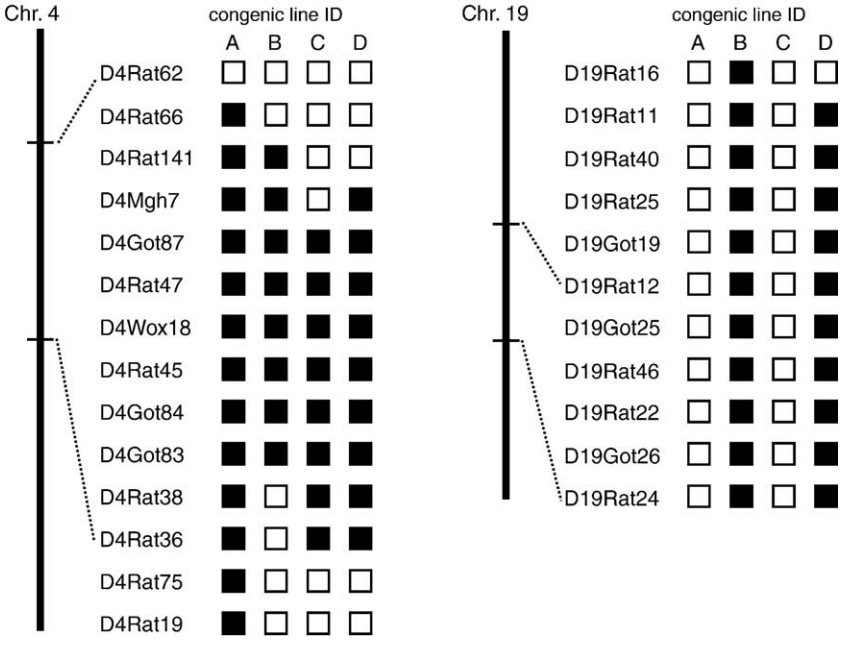

Fig. 1. Genetic analysis of rat chromosomes 4 and 19 from four congenic lines having high radiation susceptibility at the seventh generation. We illustrate the positions of the first screening makers on the rat genetic linkage map of chromosomes 4 (SRHSP × BN intercross) and 19 (FHH × ACI intercross) based on the Rat Genome Database. Black and white squares represent chromosomal segments derived from LEC and F344 rats, respectively.