Karyotypes of Rats from Strains of Different Susceptibility to Mammary Cancer Induction

E. Douglas Rees, Amy Eversole Shuck, Joseph C. Christian, and Joe R. Pugh
Departments of Medicine and Pharmacology, University of Kentucky, Lexington, Kentucky 40506

SUMMARY

The female Sprague-Dawley rat is quite vulnerable to the induction of mammary carcinomas by 3-methylcholanthrene and by 7,12-dimethylbenz(a)anthracene, a majority of the carcinomas induced are sex hormone dependent. Karyologic studies were performed on cells from rats of both strains. No difference was noted in the X or Y chromosome. In the Sprague-Dawley, both members of the #3 pair were subterminal, whereas the members were generally heteromorphic (one subterminal and one terminal) in cells from Long-Evans rats. In inbred Long-Evans rats in particular, the #12 chromosome was interesting in that one of the members frequently had larger upper arms than the other, and, not infrequently, one of the homologs had large satellites on prominent upper arms. Study of diploid cells of both strains indicated a tendency, especially after administration of 3-methylcholanthrene or 7,12-dimethylbenz(a)anthracene, for a chromosome of subterminal morphology to be missing and a terminal chromosome to be gained, apparently through loss of upper arms of the former. Karyotypes of inbred Fischer, Marshall, and Osborne-Mendel rats were also established. The present data do not indicate a correlation between karyotype and susceptibility to mammary cancer induction.

INTRODUCTION

In a study of the karyotypes of three strains of laboratory rats, Hungerford and Nowell (6) noted polymorphism of the X chromosome in the noninbred Lewis and Wistar (Shay) strains. In these two strains, the Y chromosome was the smallest terminal chromosome, whereas in the BN strain, the Y chromosome could not be distinguished from medium-sized terminal autosomes. Fitzgerald (2) previously determined a Wistar karyotype and found that one of the homologs of the #12 chromosome was the smallest terminal chromosome and the X chromosome was one of the largest terminal chromosomes (but less distinctive than the Y). The results were in accord with those obtained earlier on the laboratory rat at Lund by Tjio and Levan (14). In each of these rat strains, there were 5 subterminal, 8 terminal, and 7 median autosomal pairs. More recently, Yosida and Amano (16) reported similar findings in their karyologic studies of several strains of laboratory and wild rats; however, polymorphism of the #3 chromosome pair was noted in some strains. Bianchi and Molina have described polymorphism of the smallest subterminal chromosome in their strain of laboratory rat (1).

Sydnor et al. (13; personal communication) have demonstrated differences between rat strains in susceptibility to mammary cancer induction by oral administration of the polycyclic aromatic hydrocarbon carcinogens, 3-methylcholanthrene and 7,12-dimethylbenz(a)anthracene. In view of (a) the sex-hormone dependency of these induced mammary cancers (3), (b) the differential susceptibility of rats of different strains to form these cancers (13), and (c) the X chromosome polymorphism noted in at least one rat strain (6), a study of the karyotypes of rat strains of differing susceptibility to mammary cancer induction was initiated. The susceptible Sprague-Dawley strain and the relatively resistant Long-Evans strain were primarily emphasized in the present study, but the karyotypes of Fischer, Marshall, and Osborne-Mendel rats were also established.

MATERIALS AND METHODS

Sprague-Dawley rats were obtained from the Holtzman Company (Madison, Wisconsin). Long-Evans rats were provided by Dr. Katherine Sydnor and were all descended from a single mating pair (obtained from Diablo Farms, Inc., Berkeley, California). The animals were maintained in stainless steel cages with wire bottoms in an air-conditioned room. Water and chow pellets were available ad libitum. Liver was provided once a week and lettuce twice a week. Most of the animals were 50–100 days old at the time of the study, but younger and older animals were also studied; however, no variation in karyotype was noted with age, though older animals generally did not provide as many good spreads. Chromosomes were prepared from marrow of the femur and tibia by the method of Tjio and Whang (15) and stained with aceto-orcein. Photographs of 22 suitable spreads were obtained and enlarged to about ×5500 for chromosome measurements. At the time of karyotype construction, each chromosome in a photograph was checked against the preparation by microscopic examination. Karyotypes (Figs. 1–5) were arranged according to Hungerford and Nowell (6). The mean relative length of chromosomes and the accompanying standard deviations and standard errors were also calculated.
In the latter part of this study, chromosome spreads were prepared using the fixation procedure of Moorhead et al. (9), since a greater number of good spreads were obtained. These animals were injected intraperitoneally with 0.75 mg of colchicine in 1 ml of isotonic saline a half hour before the animal was decapitated, and the femoral and tibial marrows were removed for study. The slides were stained with aceto-orcein or with Giemsa stain. In order to minimize statistical bias in evaluating the distribution of chromosome number per cell, counts of chromosome number were limited to no more than 10 cells per rat (225 cells from 23 Sprague-Dawley rats and 137 from 14 Long-Evans rats). For the Sprague-Dawley strain, 90% of all cells were diploid; for Long-Evans, 95% were diploid. With few exceptions, deviations from diploidy were numbers smaller than 42 that probably represented an artifact of the technic mainly. The study of both rat strains was concurrent.

Since a karyotypic difference between the Long-Evans and Sprague-Dawley strains was found, additional strains of inbred rats of the Fischer, Marshall, and Osborne-Mendel strains were obtained for study from Dr. Katherine Sydnor, who had determined their susceptibility to mammary cancer induction. The karyotypic studies of these three strains were not as extensive as for the Long-Evans and Sprague-Dawley animals; but at least 10 good preparations from each of at least 5 rats of each strain were carefully examined. Also, the influence of polycyclic hydrocarbons on marrow cell chromosomes of 50-day-old female Sprague-Dawley rats was determined by studying preparations 1–16 days after intragastric administration of a single dose of either 3-methylcholanthrene (100 mg) or 7,12-dimethylbenz(a)anthracene (20 mg).

RESULTS

It is evident by microscopic examination, as well as by statistical analysis of karyologic measurements, that certain pairs of chromosomes are distinguishable and others are not. Although the largest median chromosomes are significantly larger than the shortest terminal autosomes, the karyotype system of Hungerford and Nowell (6) was followed for reasons of simplicity and in recognition of the limitation (10) of pairing by length (adjoining chromosomes in the 4–10 and 14–20 group could not be distinguished from one another). The two largest subterminal (#1 and #3) pairs, the largest terminal (#2) pair, and generally the Y chromosome were readily distinguishable in both Sprague-Dawley and Long-Evans rats. The X chromosome seemed to be the second largest terminal chromosome, but often could not be distinguished definitely. Polymorphism was not recognized in the X chromosome of either the Sprague-Dawley or the Long-Evans rat. On plotting the arm ratios of the six smallest subterminal chromosomes against their relative lengths in the manner of Patau (10), the #11 and #13 chromosomes of both strains fell into completely distinct groups, but the group of #12 chromosomes overlapped somewhat the margins of the #11 and #13 groups. Due to shorter upper arms, the #12 subterminal pairs had greater long arm/short arm ratios than did the #11 and #13 pairs. One of the #12 homologs not infrequently had larger upper arms than did the other, especially in cells from Long-Evans rats. In good preparations, each subterminal chromosome could generally be classified on careful microscopic examination. It should be mentioned that, according to the criteria and nomenclature of Levan et al. (8), the centromere of the #11 and #13 chromosomes is in a submedian rather than subterminal position; more specifically, these authors suggest that what we specify for convenience as subterminal chromosomes in the rat should be designated as smst (submedian-subterminal) chromosomes.

Most of the variability in gross chromosome morphology observed in both strains was in the subterminal chromosomes. In the Long-Evans rats, almost invariably one of the #3 homologs was terminal and the other was either satellited or had definite upper arms (Figs. 6, 7). Occasionally both members were terminal. Generally both members of the #3 pair in the Sprague-Dawley rat were subterminal; but it was not unusual for satellites, rather than definite upper arms, to be present on one or both members (Fig. 8). Rarely one of the homologs appeared to be terminal. Both members of the #11 pair were subterminal in both strains, and, in some cells of some Long-Evans rats, at least one of the homologs possessed satellites. One or both of the members of the #12 pair often had satellites rather than definite upper arms in both Sprague-Dawley and Long-Evans rats (Fig. 6). In both strains the upper arms of the #12 chromosomes were shorter, less plump, and less spread apart than those of the #11 chromosomes (Figs. 6–9). Not infrequently a single morphologically unique #12 (Fig. 7) was seen in cells from the inbred Long-Evans rat but not Sprague-Dawley rats. The general appearance of this chromosome was one of large satellites extending from prominent upper arms; sometimes a secondary constriction of the lower arms was suggested instead. We have not seen this sort of chromosome in cells from noninbred Long-Evans rats obtained from several commercial sources. Occasionally satellites were seen on one or both of the #13 subterminal chromosomes in the Sprague-Dawley rats (Fig. 9); this was noted quite often in the Long-Evans rats (Fig. 7). More recently, we have carried out karyologic studies on Sprague-Dawley rats obtained from commercial sources other than Holtzman, and we have detected no definite difference in Sprague-Dawley karyotype in these rats.

Only minor differences were observed in the karyotypes of the Osborne-Mendel, Fischer, and Marshall strains. The X chromosome(s) could frequently be distinguished in the Marshall and Fischer strain but very seldom in the Osborne-Mendel animals. The Y chromosome also was quite distinctive in the Marshall and Fischer rats, though appearing somewhat more globular in the latter. In Osborne-Mendel males, the Y chromosome could only occasionally be definitely recognized. In all three strains, both members of the #3 chromosome pair were subterminal; they were frequently satellite in the Marshall and Fischer rats. Only occasionally was a satellite chromosome noted in the marrow cells of the Osborne-Mendel rats (generally on a #11 chromosome). Satelliting was also common on #13 chromosomes in the Fischer and Marshall rats. Differences in the size of the upper arm of the #12 chromosome was not nearly so marked or frequent in these strains as in the Long-Evans animals.
Karyotypes of Rats of Different Cancer Susceptibility

Table 1

<table>
<thead>
<tr>
<th>Rat strain</th>
<th>Cells analyzed</th>
<th>Subterminal chromosomes/cell</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>10</td>
</tr>
<tr>
<td>Holtzman</td>
<td>97</td>
<td>90.7</td>
</tr>
<tr>
<td>Long-Evans</td>
<td>88</td>
<td>5.7</td>
</tr>
</tbody>
</table>

Distribution of number of subterminal chromosomes in diploid cells.

* Percent of cells.

DISCUSSION

The present study and that of Yosida and Amano (16) have only two rat strains in common, the Long-Evans and Fischer. With respect to the Fischer, the observations on the #3 chromosome pair were the same: both members were subterminal. In the case of their inbred Long-Evans strain, both members of the #3 pair were subterminal, whereas the strain we used had a heteromorphic pair. Yosida and Amano (16) felt that the X chromosome is intermediate in size between the fourth and fifth largest chromosome pairs; our observations are more in accord with those of Hungerford and Nowell (6), who placed the X between the #3 and #4 pairs. It is difficult to be certain on this point, however, for in many instances the X chromosome cannot be distinguished definitely.

In most good chromosome preparations it is possible to identify the morphology of each chromosome and to determine the number of chromosomes in each morphologic group. Counting the number of chromosomes in each morphologic group permits a convenient analysis for gross chromosome alterations, since the procedure can be done directly under the microscope and the results can be expressed quantitatively. Variation of the number of chromosomes in each morphologic group can be due to actual karyologic differences, to an artifact of preparation, and to an error in assigning a morphologic classification. In any case, it is important to know for comparative purposes the magnitude of variation in normal cells. Approximately 10 percent of the diploid cells in Sprague-Dawley rats showed an alteration in the morphologic grouping, and generally this involved a missing subterminal chromosome (Table 1) with corresponding gain of a terminal chromosome. Intragastric administration of 3-methylcholanthrene or 7,12-dimethylbenz(a)anthracene more than doubled the incidence of morphologic alterations in diploid cells (Table 2), and, again, the primary alteration was loss of a chromosome of subterminal morphology and gain of a terminal chromosome. Presumably this represents loss of the upper arms of a subterminal chromosome with conversion to terminal morphology. In the untreated rats, a #3 chromosome was predominately involved in this change, whereas with 3-methylcholanthrene or 7,12-dimethylbenz(a)anthracene treatment, the other subterminal chromosomes were mainly affected. Although the technic of chromosome preparation for cultured cells differs from that for marrow cells, it is of interest that diploid cells cultured from induced mammary carcinomas were found to have a diminished number of subterminal chromosomes (11; unpublished studies). A nonrandom representation of chromosome types in human tumor stemlines has been noted by Levan (7) and Steenis (12).

The female Long-Evans rat is relatively resistant (13) to induction of mammary cancer by intragastric instillation of 3-methylcholanthrene or 7,12-dimethylbenz(a)anthracene as compared to the marked susceptibility of the female Sprague-Dawley rat. On the other hand, the incidence of leukemia is much higher in Long-Evans rats than in Sprague-Dawley rats after intravenous administration of 7,12-dimethylbenz(a)anthracene (4, 5). A relationship between heteromorphism of the #3 chromosome pair in the Long-Evans rat and its response to the polycyclic hydrocarbon carcinogens was considered, though it did not seem likely. Any simple relationship is ruled out, however, by the study of the Fischer, Marshall, and Osborne-Mendel karyotypes and the observation that intragastric instillation of a single dose of 7,12-dimethylbenz(a)anthracene (100 mg/kg body weight) induces mammary cancers in 90-100% of female Sprague-Dawley and Osborne-Mendel rats but in only about 10% of Long-Evans, Fischer, and Marshall rats (Katherine Sydnor, personal communication). The only other karyotypic difference noted between the strains was the presence of a morphologically unique #12 chromosome which was seen not infrequently in cells from Long-Evans rats. No apparent difference in the sex chromosomes of these two rat strains was noted. On the basis of present data there seems to be no direct relationship between strain karyotype and tumor susceptibility.

ACKNOWLEDGMENTS

We express our thanks to Dr. Katherine Sydnor who generously supplied the inbred rats and provided data on the tumor susceptibilities of the different rat strains. Dr. Peter C. Nowell kindly reviewed some of our slides and offered helpful advice.

REFERENCES


Karyotypes of Rats of Different Cancer Susceptibility

Holtzman

1 2 3
10

X,4

11 12 13 Y

2

Long-Evans

1 2 3
10

X,4

11 12 13 Y

2

May 1968
Fig. 1. Karyotype of male Sprague-Dawley (Holtzman) rat.
Fig. 2. Karyotype of male Long-Evans rat.
Fig. 3. Karyotype of male Fischer rat.
Fig. 4. Karyotype of male Marshall rat.
Fig. 5. Karyotype of male Osborne-Mendel rat.
Fig. 6. Metaphase chromosome of female Long-Evans rats arrow indicates satellited #12 chromosome. Giemsa, X 2000.
Fig. 7. Metaphase chromosomes of female Long-Evans rats upper arrow indicates a #12 chromosome with large satellites on prominent upper arms, and lower arrow indicates a satellited #13 chromosome. Giemsa, X 2400.
Fig. 8. Metaphase chromosomes of female Sprague-Dawley rat; a satellited #3 chromosome is indicated by arrow. Giemsa, X 1700.
Fig. 9. Metaphase chromosomes of female Sprague-Dawley rat; arrow points to satellited #13 chromosome. Giemsa, X 1800.
Karyotypes of Rats from Strains of Different Susceptibility to Mammary Cancer Induction


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/28/5/823

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.