Karyotypic Changes Associated with Loss of Prolactin Dependency of Rat Nb2 Node Lymphoma Cell Cultures

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ABSTRACT

The parent line of cultured "Nb2 node" lymphoma cells is dependent on the hormone prolactin (PRL) for growth and is widely used for the in vitro bioassay of lactogenic hormones. As reported previously, PRL-independent sublines have been developed in vitro from the parental line by lactogen deprivation. The present study describes the G-banded karyotypes of the Noble (Nb) strain of rat (in which the original lymphoma developed), the PRL-dependent cell line (157th generation), and two of its PRL-independent sublines (1220th and 2372nd generations). The karyotype of the Nb rat was determined to be the same as that of Rattus norvegicus. The stemline karyotype of the PRL-dependent cells contains a number of well-defined chromosomal abnormalities. The PRL-independent sublines examined have the same chromosomal abnormalities as the PRL-dependent cells plus a few additional changes indicative of clonal evolution from the PRL-dependent stemline. The development of PRL independence (as seen in the 1220th generation) was associated with only two karyotypic changes, i.e., loss of the Y chromosome and a translocation involving chromosomes 14 and 17. The recently reported mapping of the rat PRL gene and other PRL-related genes to chromosome 17 suggests that rearrangement of chromosome 17 could be involved in the development of the PRL independence. The PRL-dependent and PRL-independent Nb2 cell lines provide a useful system for studying chromosomal and molecular genetic events associated with the malignant progression of polypeptide hormone-dependent cancers.

INTRODUCTION

Tumor progression is a characteristic feature of malignant cancers and is manifested by alterations in phenotypic behavior such as increases in virulence, loss of dependence on growth factors, development of drug resistance, tissue invasiveness, and metastatic ability (1, 2). The progression proceeds in a stepwise manner (3) and involves the clonal development in the cancers of variant sublines which arise, at least in part, from enhanced genetic instability (1, 2). Cytogenetic studies of human leukemias and lymphomas have demonstrated that progression of these diseases is associated with the sequential acquisition of nonrandom chromosomal changes (4). There is evidence that such changes are critical steps in the development and progression of many other types of cancer (5, 6).

Hormone-dependent cancers such as may originate in the breast, prostate, and endometrium are commonly treated with hormone withdrawal therapy to take advantage of their dependency on specific hormones for growth. While this type of therapy may produce long-lasting remissions, disease relapse frequently occurs in a more malignant form which is not hormone dependent and hence fails to respond to further endocrine treatment. Such tumor progression is a major problem in the clinical management of these cancers (7).

The PRL-dependent rat "Nb2 node" lymphoma cell line has provided a useful system for studying the in vivo and in vitro growth characteristics of polypeptide hormone-dependent cancers and their progression toward hormonal independence (for reviews see Refs. 8–10). The cell line was established in culture from a transplant of a malignant lymphoma that arose in an estrogenized male Nb rat (8, 11). It has been determined to have the phenotypic characteristics of an early thymocyte (11, 12). The cultured cell line has retained principal properties of the parent tumor, including a specific requirement for PRL (or other lactogens) for growth and the ability to give rise to PRL-independent tumors when injected s.c. into Nb rats (9). The specific growth requirement of the cultures for PRL has led to its extensive use for the in vitro bioassay of lactogenic hormones (8–10). When subcultured in medium which is deficient in lactogens, most of the cells die within a few days; some cells survive, however, and give rise to sublines which no longer require exogenous PRL for growth (13). These PRL-independent cells are cytologically very similar to the parent cells but have a higher proliferation rate; they are also tumorigenic (13).

We have initiated a cytogenetic investigation to determine if there are karyotypic differences between PRL-dependent and PRL-independent Nb2 node lymphoma cell lines which can be correlated with their difference in PRL dependency. In this study karyotypes have been established of the parent PRL-dependent cell line and of two PRL-independent sublines previously derived from the parent line by continuous lactogen deprivation (Fig. 1; Ref. 13). Evidence has been obtained that the PRL-independent lines have clonally evolved from the PRL-dependent stemline with only minor chromosomal changes which may be nonrandom and potentially important in determining the chromosomal regions and genes involved in the development of PRL independence.

MATERIALS AND METHODS

Animals

Male Nb rats (weight ca 200 g) were used as a source of normal Nb rat cells. The animals were obtained from the colony of Nb rats which is maintained by random littermate breeding (14) at the British Columbia Cancer Research Centre.

Nb2 Node Lymphoma Cell Lines

Cultured PRL-dependent and PRL-independent Nb2 node lymphoma cell lines were revived from liquid nitrogen storage for chromosome analysis. Fig. 1 shows how the three lines are related and gives an indication of their age expressed in generations (i.e., the number of population doublings that have occurred in vitro since the parent line was established in culture). The cell lines are designated: (a) Nb2-U17, the parent noncloned PRL-dependent cell line (157th generation); (b)
Cell Culturing

Normal Nb Rat Fibroblasts. Cultures of normal rat fibroblasts were established from freshly minced pieces of fascia, lung, or peritoneum isolated from healthy male Nb rats and incubated at 37°C in N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid-buffered RPMI supplemented with FBS (20%), L-glutamine (20 μM), and antibiotics. For subculturing, the fibroblasts were detached by incubation with 0.05% trypsin plus 0.02% EDTA for 2 min at 37°C.

Normal Nb2 Node Lymphoma Cells. Cells, resurrected from liquid nitrogen storage, were maintained in suspension culture following procedures described previously (13). Briefly, the PRL-dependent cells were incubated in Fischer’s medium supplemented with 10% FBS (a source of lactogen), lactogen-deficient horse (gelding) serum (10%), 2-mercaptoethanol (0.1 mm), penicillin (50 units/ml), and streptomycin (50 μg/ml) at 37°C in an atmosphere of 5% CO2–95% air. The PRL-independent Nb2 line and its various sublines, as distinct from the parental U17 line, can proliferate readily in lactogen-free, chemically defined medium (13).

Chromosome Preparations

Normal Nb rat fibroblasts (1st or 2nd passage) were subcultured onto Petri dishes and incubated for 24 h at 37°C. Metaphases were harvested in situ by a 30-h incubation with Colcemid (0.01 μg/ml; Gibco), a 30-min hypotonic treatment (1% sodium citrate), and fixation with methanol:acetic acid (3:1). The dishes were air dried and stored overnight at 56°C. Metaphases were banded by a 30-60-s exposure to Giemsa, a 30-min hypotonic treatment (1% sodium citrate), and fixation with methanol:acetic acid. The number of chromosomal abnormalities present in the PRL-dependent Nb2-U17 stemline was relatively low (i.e., five; Table 1). At the 300–400 band resolution level it was possible to determine the chromosomal rearrangements and the breakpoint sites quite precisely. The chromosomal changes may have arisen in the animal during the development of the malignant lymphoma and subsequent serial transplantation and/or in vitro during subculturing. The extent to which the chromosomal changes involve cancer-specific genes or are the result of random chromosome breakage or rearrangement has yet to be determined. In this regard, however, it is of interest that at least three of the breakpoint sites in the Nb2-U17 cells (i.e., 2q22,
4q24, and 18p12) are identical to common fragile sites expressed in the genome of the laboratory rat (19). The significance of such fragile sites in rat malignancies is uncertain, since little or no apparent concordance has been established between the observed common fragile sites and cancer-specific break-point sites found in spontaneous and chemically or virally induced rat cancers (19, 20). The results of the present study, however, do not exclude the possibility that some of the fragile sites may be involved in the development of lymphomas, at least in estrogenized Nb rats (8).

The observation that all the chromosomal abnormalities of the parental PRL-dependent Nb2-U17 stemline are also present in the PRL-independent PRA and SFJCD1 sublines (Table 1) indicates that these cell lines have common clonal ancestry. The additional karyotypic changes in the sublines suggest that the three Nb2 cell lines have developed along a common evolutionary pathway in which the SFJCD1 line has clonally evolved.

Fig. 2. G-banded karyotype of normal male Nb rat fibroblasts.

Fig. 3. G-banded karyotype of the PRL-dependent Nb2-U17 lymphoma cells (157th generation). Bars, absent chromosomes; arrows, reciprocal translocations; arrowheads, derivative chromosomes.
evolved from the PRA line which in turn clonally evolved from the U17 stemline (Table 1).

The karyotypic changes in the examined Nb2 cell lines are associated with progression-related phenotypic changes, i.e., the development of PRL independence and increased growth rate (Table 1). In the case of the PRL-dependent U17 and PRL-independent PRA cell lines only two karyotypic differences were found, i.e., $-Y$ and $t(14;17)$, raising the question as to whether there is a causal relationship between these karyotypic changes and, e.g., the development of PRL independence. It is not known if the loss of the Y chromosome plays a significant role in the loss of the PRL dependency. (In humans, loss of the Y chromosome has been observed in certain leukemias and lymphomas, but there is no unequivocal evidence that it is of
etiological or prognostic significance (21); there is evidence that it may be a normal variant of cell phenotyping (22). On the other hand, abnormalities affecting chromosome 17 could have a role in the development of the PRL independence in the Nb2 cell lines, since somatic cell hybridization studies have indicated that, in the rat, chromosome 17 harbors the PRL gene (23) and also a number of PRL-related genes, i.e., the genes for placental lactogen II and PRL-like proteins A and B (24). In the PRL-dependent Nb2-U17 cells there is monosomy for chromosome 17; in the PRL-independent PRA and SFJCD1 cells the remaining chromosome 17 is modified by a translocation with chromosome 14 (Figs. 3–5; Table 1). In view of these findings it will be of interest to determine if the PRL dependency of the Nb2 cells is related to the loss of one chromosome 17 and if the t(14;17) has led to a disturbance in the function of PRL-related genes (including the PRL receptor gene) which may underlie the development of PRL independence. In this connection it may be noted that the PRL independence of the PRA and SFJCD1 cells does not appear to be due to their production of mitogenic autocrine or intracellular PRL-like factors, since neither extracts of these cells nor medium conditioned by them had activity in the Nb2 cell bioassy (13).

The present study has shown that the progression of the Nb2-U17 line to PRL independence (Fig. 1) has involved the clonal emergence of variant PRL-independent sublines which became dominant during lactogen deprivation as the PRL-dependent cells perished. It is not known if the variant sublines preexisted in the original U17 culture as minor subpopulations or if they developed as a result of lactogen deprivation. The progressive chromosomal changes seen in these cell lines resemble those observed in other animal and human cancers (1, 2, 25–28) suggesting that the Nb2 cell lines are useful for studying chromosomal and molecular genetic events associated with the malignant progression of polypeptide hormone-dependent cancers.

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**Table 1 Karyotype and progressive changes found in related Nb2 node lymphoma cell lines**

<table>
<thead>
<tr>
<th>Cell line*</th>
<th>Karyotype</th>
<th>Progressive changes</th>
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<tbody>
<tr>
<td>Parent U17, PRL-dependent, DT = 20 h</td>
<td>42,XY,−17t(2;4)(q22;q24), t(10;11)(q12;p12), +der(13)(p15.7)(p12.2), −18,+der(18)t(18;7)(p12;7)</td>
<td>−Y</td>
</tr>
<tr>
<td>PRA, PRL-independent, DT = 17 h</td>
<td>41,X,Y,−17t(2;4)(q22;q24), t(10;11)(q12;p12),t(14;17)(p22;p14), +der(13)(p15.7)(p12.2), −18,+der(18)t(18;7)(p12;7)</td>
<td>t(14;17)</td>
</tr>
<tr>
<td>SFJCD1, PRL-independent, DT = 14 h</td>
<td>40,X,Y,−17,2inv(1)(q31;q41), t(2;4)(q22;q24), t(10;11)(q12;p12), −11t(11;11)(p11;7), −13,+der(13)(p13;7), t(14;17)(p22;p14), −15,+der(15)(p15;7)(p12;7), −18,+der(18)t(18;7)(p12;7)</td>
<td>inv(1q) 11p+ 13p+ −15</td>
</tr>
</tbody>
</table>

* Phenotypic properties, including culture-doubling time (DT), as described in Ref. 13.

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**REFERENCES**

23. Cooke, N. E., Szpirer, C., and Levan, G. The related genes encoding growth
hormone and prolactin have been dispersed to chromosomes 10 and 17 in the rat. Endocrinology, 119: 2451–2454, 1986.
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