Cytogenetic Alterations Associated with the Acquisition of Doxorubicin Resistance: Possible Significance of Chromosome 7 Alterations

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ABSTRACT

Cytogenetic abnormalities associated with the acquisition of doxorubicin (DOX) resistance (DOXR) were examined in two cell lines (HT1080 fibrosarcoma and LoVo colon adenocarcinoma) which were selected in the presence of increasing concentrations of DOX over a 2-year period. Karyotypes of both tumor lines were initially near-diploid although they differed significantly in their intrinsic sensitivities to DOX (DOX 50% inhibiting concentrations: LoVo, 0.10 µg/ml; HT1080, 0.006 µg/ml). Chromosome banding analysis of DOXR sublines of the LoVo and HT1080 cell lines demonstrated a strikingly different response to DOX selection with regard to both numeric and structural chromosome alterations. DOXR LoVo cells maintained the parental modal chromosomal number of 49 despite a 285-fold increase in the level of resistance, with minimal structural chromosome changes observed. In contrast, the development of DOXR in HT1080 cells was accompanied by marked aneuploidy, including a significant increase in the complexity of the tumor karyotype with increasing levels of DOXR. Cytogenetic evidence suggestive of gene amplification (double minutes and homogeneously staining regions) was also observed in the DOXR HT1080 cell line. Examination of chromosome alterations common to both resistant lines revealed alterations of chromosomes 1, 5, 7, and 11, with chromosome 7q the most frequent site of chromosome change. Reversion of DOXR in both the HT1080 and LoVo cell lines (by continuous in vitro passage once off drug) resulted in an accompanying loss in structurally altered No. 7 chromosomes. Our data suggest that alterations of chromosome 7 are a common and perhaps significant feature of DOXR tumor cells.

INTRODUCTION

DOXR (Adriamycin) is the principal therapy for many forms of human cancer (1, 2). Unfortunately, DOX treatment is often limited by the development of drug resistant cell populations, which characteristically display collateral (or multiple drug) resistance to a wide range of amphiphilic, heterocyclic anticancer agents (3). The acquisition of this MDR phenotype has been correlated with several biological phenomena, most notably the overexpression of a M, 150,000-180,000 glycoprotein (termed P-glycoprotein) (3-10). The principal focus of this paper will be to compare the chromosome changes accompanying the development of doxorubicin induced MDR.

Cytogenetic analyses of DOXR tumors are limited, with most previously reported cases examining rodent tumors (11-15). The specific objective of the present study was to cytogenetically characterize two recently developed DOXR human tumor cell lines, LoVo (a colon carcinoma cell line) and HT1080 (a fibrosarcoma cell line). These cell lines were chosen for this study because of their near-diploid karyotypes and their very different intrinsic sensitivities to DOX (IC50: LoVo, 0.10 µg/ml; HT1080, 0.006 µg/ml). As described below, these two tumor cell lines were strikingly different in their patterns of chromosome alterations that followed a similar DOX selection schema.

RESULTS

The following sections will describe and contrast the clonal numeric and structural chromosome alterations which accompanied the acquisition of DOXR for both the LoVo and HT1080 cell lines. The data will first be summarized with regard to numeric chromosome alterations followed by a description of structural chromosome alterations unique to the resistant sublines.

Numeric Chromosome Alterations Accompanying DOXR. Parallel cytogenetic studies of the parental and DOXR LoVo and HT1080 sublines were performed at presellected drug levels (Table 1). The pattern of numeric chromosome alteration which
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Fig. 1. Histograms indicating the number of chromosomes per cell from LoVo (left) and HT1080 (right) parental and DOX-resistant sublines. At least 30 metaphases were analyzed per subline. The percentages of cells at a particular chromosome number are indicated in the left column for the LoVo parental (A) and DOX-resistant (DR4 DR) sublines and in the right column for the parental (G) and HT1080 DOX-resistant (DR3 DF) sublines. Note that the modal chromosome number remains essentially constant during the acquisition of DOX resistance in the various LoVo sublines, while in contrast, HT1080 DOX-resistant sublines displayed significant numeric changes. F and L, DOX-resistant sublines carried free of drug (LoVo/DR4; HT1080/DR3 DF).

accompanied the increasing levels of DOX-resistant levels varied significantly between these two cell lines (Fig. 1). LoVo parental cells were characterized by a modal chromosome number of 49 which remained stable over the entire 2-year selection period (Fig. 1A). The modal chromosome number and range of chromosomes per cell remained essentially constant (with some occasional hyperdiploid cells) despite an increase in the level of DOX to 285-fold control (Fig. 1, B–E). In contrast to the stability of the modal chromosome number of the LoVo parental line, the pseudodiploid stemline of the HT1080 parental line (Fig. 1G) was shown following serial in vitro passage without drug to be almost entirely replaced by cells in the near-tetraploid range. In further contrast to the LoVo cell line, the acquisition of DOX resistance in HT1080 cells was also accompanied by prominent changes in chromosome number (principally hyperdiploidy) (Fig. 1, H–K).

Both the LoVo/DR4 (LoVo/DR4 DF) and the HT1080/DR3 (HT1080/DR3 DF) sublines were grown in the absence of DOX to assess the stability of the DOX-resistant phenotype and to correlate the stability of the observed karyotype alterations. After 5 months (~90 population doublings) with no drug exposure, the LoVo/DR4 subline returned to a level of drug resistance approximating the parent, demonstrated a near-diploid chromosome number, and maintained a range around the mode essentially identical to that of the parental line (Table 1; Fig. 1F). In contrast, the HT1080/DR3 subline quickly lost its drug-resistant phenotype and shifted to a near-tetraploid chromosome number (after ~1 month and ~30 population doublings) (Table 1; Fig. 1L). This tendency toward replacement of the diploid population with near-tetraploid cells as mentioned previously was also observed for the parental line carried continuously in cell culture.
Table 1 DOX resistance and cytogenetic characteristics in the various HT1080 and LoVo sublines

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Selective concentration (µg/ml)</th>
<th>Relative resistance</th>
<th>Cytological evidence of gene amplification (Y/N)/type</th>
<th>Modal chromosome no.</th>
</tr>
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<tr>
<td>LoVo</td>
<td>0</td>
<td>1.0</td>
<td>N</td>
<td>49</td>
</tr>
<tr>
<td>LoVo/DR1</td>
<td>0.025</td>
<td>10.0</td>
<td>N</td>
<td>49</td>
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<tr>
<td>LoVo/DR2</td>
<td>0.05</td>
<td>15.0</td>
<td>N</td>
<td>49</td>
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<td>28.0</td>
<td>N</td>
<td>49</td>
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</tr>
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<td>285.0</td>
<td>N</td>
<td>49</td>
</tr>
<tr>
<td>LoVo/DR4 DFa</td>
<td>0</td>
<td>1.8</td>
<td>N</td>
<td>49</td>
</tr>
<tr>
<td>HT1080</td>
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<td>1.0</td>
<td>N</td>
<td>46</td>
</tr>
<tr>
<td>HT1080/DR1</td>
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<td>16.0</td>
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</tr>
<tr>
<td>HT1080/DR2</td>
<td>0.025</td>
<td>30.0</td>
<td>Y/DMs+b</td>
<td>75</td>
</tr>
<tr>
<td>HT1080/DR3</td>
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<td>92.0</td>
<td>Y/DMs+b</td>
<td>71</td>
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<tr>
<td>HT1080/DR4</td>
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<td>Y/DMs+HSR</td>
<td>69</td>
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<tr>
<td>HT1080/DR3 DFb</td>
<td>0</td>
<td>1.8</td>
<td>Y/DMs+HSR</td>
<td>86</td>
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</tbody>
</table>

aIC of the resistant sublines/IC of the parental cells.
bThe LoVo/DR4 DF subline was grown in drug free medium for 175 days.
cDM, double minute.
dThe HT1080/DR3 DF subline was grown in drug free medium for 30 days.
eOnly 2 metaphases contain 1-2 DMs/cell.

Structural Chromosome Alterations Accompanying DOXR.

Specific chromosome alterations accompanied the acquisition of DOXR. In general, structural chromosome alterations in the LoVo cell line (both the parental and DOXR sublines) were minimal and remained relatively stable, while the DOXR HT1080 sublines increased markedly in the complexity of the karyotype associated with increasing levels of DOXR.

The parental LoVo cell line demonstrated a stemline karyotype of 49XY,-2,+der(2)(2;12)(q21;p12),+5,+7,+del(12)(q14q22),-15,+der(15)(15;15)(p11;q11), with 4 sidelines observed which differed from the main stemline by the following changes: sd1+12; sd2-2Y; sd3-3; sd4-4. Selection in DOX to a level of relative resistance of ~285-fold (LoVo/DR5) resulted in the appearance of several clonal chromosome alterations unique to this resistance subline (Fig. 2B). The stem line karyotype for the LoVo/DR5 was: 49XY,-2,+der(2)(2;12)(q21;p12),+5,+inv(7)(p13q22), inv dup(8)(q11-q24::q24-q11),-15, +der(15)(15;15)(p11;q11) with 4 sidelines observed which differed from the stem line by the following changes: sd1 invdup(8); sd2 t(7;11)(q21q32); sd3 Y; sd9 t(8;11)(q24;q13) (Fig. 2B).

The parental HT1080 cell line demonstrated a stemline karyotype of 46XY,-5,+der(5)(5;7)(p15;q7),-11,+der(11)(3;11)(qter-3q12::11q25-11pter), with 6 -invdup(8); sd7 +del(7)(q21q32); sd8 Y; sd9 t(8;11)(q24;q13) (Fig. 2B).

The parental HT1080 cell line demonstrated a stemline karyotype of 46XY,-5,+der(5)(5;7)(p15;q7),-11,+der(11)(3;11)(qter-3q12::11q25-11pter), with 6 -invdup(8); sd7 +del(7)(q21q32); sd8 Y; sd9 t(8;11)(q24;q13) (Fig. 2B).

The parental HT1080 cell line demonstrated a stemline karyotype of 46XY,-5,+der(5)(5;7)(p15;q7),-11,+der(11)(3;11)(qter-3q12::11q25-11pter), with 6 -invdup(8); sd7 +del(7)(q21q32); sd8 Y; sd9 t(8;11)(q24;q13) (Fig. 2B).

The parental HT1080 cell line demonstrated a stemline karyotype of 46XY,-5,+der(5)(5;7)(p15;q7),-11,+der(11)(3;11)(qter-3q12::11q25-11pter), with 6 -invdup(8); sd7 +del(7)(q21q32); sd8 Y; sd9 t(8;11)(q24;q13) (Fig. 2B).

Fig. 3B demonstrates the significant increase in the complexity of the karyotype of HT1080 cells selected in DOX to a level of relative resistance ~30-fold over the parental line (HT1080/ DR2). Identifiable chromosome changes within the DOXR HT1080/DR2 subline included: del(1)p13, t(3;10)(q11;p11), del(5)(q22q34), del(der(5))(q22q34), i(5q), del(7)(q21q31), der(10)(4;10)(p4pter-q11::10q21-10q26::10q26-10q11), t(10;11)(q11;p11), del(11)(q13), t(13;?)(p11?), t(13;13)(p11;q11), t(14;14)(p11;q11), del(19)(3;11;19)(3qter-3q12::11q25-11q13::19q13-19pter), del(20)(q11) (Fig. 3B).

Fig. 4 presents the proposed sequence of clonal karyotypic evolution in both the LoVo and HT1080 DOXR sublines. Of interest, the type of chromosomal alterations varied between the two cell lines, with translocations and inversions the most common cytogenetic abnormalities observed in the LoVo DOXR sublines, whereas in the HT1080 DOXR subline, deletions were most frequent in the early steps of selection while translocations were most frequent at the highest levels of DOX.

When all chromosomal changes were analyzed for both the LoVo/DOXR and HT1080/DOXR sublines, four chromosomes (chromosomes 1, 5, 7, and 11) were shown to be most frequently involved in structural chromosome alterations. These alterations included chromosome translocations, deletions, inversions, and the presence of a putative HSR. The single most frequently altered chromosome was chromosome 7. Pictorial documentation of structural alterations of chromosome 7 which accompanied the acquisition of DOXR for both the HT1080 and LoVo cell lines is presented in Fig. 5. Although neither the LoVo nor HT1080 parental cell lines evidenced structural alterations of chromosome 7, by the second step of selection chromosome 7 alterations were present in both lines. Selection at increasing DOX concentrations resulted in an associated increase in the number of chromosome 7 changes (Fig. 5). The distribution of breakpoints along chromosome 7 was variable, although the preponderance of breaks occurred between band regions 7q21-q36. [Of interest, the P-glycoprotein gene in...
normal cells has been localized within this chromosomal region (18) (see “Discussion”). All copies of structurally altered No. 7 chromosomes were lost from the HT1080 and LoVo sublines which reverted to drug sensitivity after being passaged continuously in vitro with no drug exposure.

Cytological evidence of gene amplification (i.e., double minutes or HSRs) have frequently been reported in DOX\(^\text{R}\) cell lines (5, 11-15, 19-21). Examination of the LoVo/DOX\(^\text{R}\) sublines failed to demonstrate cytological evidence of amplification at any level of DOX\(^\text{R}\). In contrast, chromosomal instability (dicentrics, rings, chromosomal pulverization, etc.) was a common feature of HT1080/DOX\(^\text{R}\) sublines (even at the earliest steps of DOX\(^\text{R}\)) with both double minutes and a putative HSR (involving chromosome 7) observed at the highest level of DOX\(^\text{R}\) (HT1080/DR4) (Fig. 6).

**DISCUSSION**

The mechanism by which DOX kills cells is suggested to involve DNA intercalation and subsequent inhibition of DNA replication related functions (2). It is therefore not surprising that in normal cells acute DOX exposure results in significant chromosome damage, including breaks, gaps, dicentrics, pul-
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Fig. 4. Proposed sequence of clonal karyotypic evolution during the acquisition of DOX\textsuperscript{R} for the LoVo (upper) and HT1080 (lower) sublines. The indicated chromosome alterations represent deviations from the stemline of the parental and DOX\textsuperscript{S} sublines (see Table 1). The circles represent the size of the cell population, with O representing the clonal chromosomal alterations present in the stemline.

verization, and an increase in sister chromatid exchange (22-24). In the current study, we have examined the cytogenetic effects of chronic exposure to DOX in two human tumor cell lines: HT1080, which initially was extremely sensitive to the cytotoxic effects of DOX (IC\textsubscript{50} 0.006 μg/ml); and LoVo, which initially was quite resistant to this drug (IC\textsubscript{50} 0.10 μg/ml). It is of interest, then, that the two tumor cell lines had strikingly different karyotypic responses to acquired DOX\textsuperscript{R}, which we speculate might be partially accounted for by the observed differences in their “inherent” sensitivities to the cytotoxic action of DOX. Currently, it is not clear whether the karyotypic differences relate to possible differences in the cellular pharmacology of DOX between these cell lines (e.g., drug metabolism, intracellular drug distribution, etc.) although pharmacokinetic studies are currently under way which may help answer this question.

Structural rearrangements common to both the LoVo and HT1080 DOX\textsuperscript{R} cell lines included chromosomes 1, 5, 7, and 11. However, the most consistently altered single chromosome in both independently selected lines was chromosome 7. These results are of possible interest for two reasons: (a) nonrandom chromosomal alterations (deletions and translocations) involving chromosomes 5q and 7q have been described repeatedly in tumor cells from patients with “treatment or mutagen associated” leukemia (tumors which are highly refractory to antineoplastic therapy) (25-28); (b) the clustering of chromosomal deletions and rearrangements to the long arm of chromosome 7 is coordinate with the loci of the P-glycoprotein (MDR1) gene in normal cells (7q21-31) (18). Preliminary studies have failed to demonstrate a rearrangement of P-glycoprotein sequences (recognized by Southern blotting) associated with chromosome 7 alterations in either the LoVo or HT1080 DOX\textsuperscript{R} sublines.\textsuperscript{6} However, despite the lack of evidence of structural alteration of the MDR gene, we have demonstrated by Northern blotting the overexpression of P-glycoprotein in mRNA in the LoVo DOX\textsuperscript{R}, but not the HT1080 DOX\textsuperscript{R} cell lines.\textsuperscript{6}

In addition to alterations involving the long arm of chromosome 7, both the HT1080 and LoVo DOX\textsuperscript{R} sublines also

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Fig. 5. Chromosome 7 abnormalities in DOX® LoVo and HT1080 sublines. Pictorial documentation of chromosome 7 alterations in the LoVo/DOX® sublines included: a, inv(7)(p13;p22); b, dic(7) (pter—q36::q36—>pter); c, dic(7) (7pter—q36::q36—>pter; t(1;7)(p34;q11); e, (7;10)(q11;q26); f, dup(7) (q21-q32); g, t(7;17)(q22;q23); h, t(5;7)(q22;q32); and i, del(7)(q21q32). Pictorial documentation of chromosome 7 alterations from the HT1080/DOX® sublines included: j, del(7)(q21q31); *, inv(7)(p13;p22); and /, der(7)(q12—HSR—>q22::q22). Bottom, Idiogram of G-banded chromosome 7 depicting breakpoints involved in structural rearrangements in both the LoVo and HT1080 cells. The percentage of breakpoints observed on the 7q arm was 74% with 32% observed near or at the loci of the P-glycoprotein gene (P-gly), cystic fibrosis gene (CF); and c-met cellular oncogene. Breakpoints along 7p were also observed coordinate with the loci of the epidermal growth factor receptor gene (EGFR).

Fig. 6. Various cytogenetic abnormalities observed in HT1080 DOX® cells. A, endoreduplication; B, chromosome 7 abnormalities, fragments, dicentrics, and a ring chromosome; C, double minutes; D, der(7) HSR.

demonstrated structural alterations of the short arm of chromosome 7 (p13—>p22). This region of 7p is coincident with the chromosomal loci of the epidermal growth factor receptor gene (29). Recent reports have suggested that epidermal growth factor receptor expression is significantly elevated in MDR cell lines (30), while other reports have demonstrated that DOX exposure itself may modulate the expression of epidermal growth factor receptor in HeLa and murine 3T3 cells (31).

This study demonstrates that as cells acquire DOX® they may also concordantly display recurring sites of chromosome change. Studies are now under way to examine the possible role of these chromosome alterations in modulating the expression of P-glycoprotein for alternate genes which may be important in the acquisition of DOX®.

REFERENCES


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