Chromosome Changes Characterizing in Vitro Response to Radiation in Human Squamous Cell Carcinoma Lines

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ABSTRACT

We have analyzed the karyotypes of a series of 16 cell lines derived from human squamous cell carcinomas of the head and neck. When the cell lines were grouped according to their in vitro response to radiation, it was observed that some recurrent chromosomal changes occurred with differing frequencies between groups. Radiation resistance was associated with clusters of breakpoints in bands 1p22, 3p21, and 8p11.2 and deletion of distal 14q, while relative radiation sensitivity was associated with a high frequency of breakpoints in 11q13 and duplication of distal 14q. The regions identified point to the possible locations of genes involved in the response to radiation and could provide a series of markers with which to predict response to radiation therapy.

INTRODUCTION

Cytogenetic analyses of solid tumors have shown that in some types of tumor recurrent chromosome changes can be identified, and these can be used as an aid in differential diagnosis of the tumor (1). Karyotype changes also have been correlated with outcome (2). For the majority of solid tumors, however, the karyotype changes observed are complicated with many unbalanced translocations but no specific tumor translocation. The unbalanced translocations result in a loss and gain of whole or parts of many chromosomes. The duplications and deletions are not random but rather indicate the chromosomal position of potential tumor suppressor genes (deletions) or oncogenes (duplications) involved in tumorigenesis.

HNSCC3 cell lines and cells from primary cultures have shown that the majority have complex karyotypes with typically more than 50% of chromosomes in any karyotype involved in rearrangement (reviewed in Ref. 3). Recurrent deletion of parts or all of chromosome numbers 1, 3, 4, 7, 8, 9, 10, 11, 13, and 18 have been reported (3–12). Clusters of translocation breakpoints have been reported in chromosome 1 band 1p22 and chromosome 11 band q13 (3, 13).

The karyotype changes seen in HNSCC show similarity to those seen in esophageal and lung tumors with deletion of 3p being a change common to all three tumor types (14–16). As tobacco use has been implicated in the etiology of all of these tumors, the observations suggest that chromosome 3p may be the site of a gene preferentially affected by a component of tobacco smoke. Additionally, amplification of a group of putative oncogenes on chromosome 11q13, HST1, BCLI, INT2, and PRAD1, has been reported in HNSCC and esophageal cancer (17–20).

Despite emphasis on improving early diagnosis, survival rates for patients with head and neck tumors have not improved dramatically in recent years. One major problem in designing treatment protocols is the spontaneous occurrence of radiation resistance, resulting in the failure to achieve local control of disease. Investigators report that, overall, in vitro radiation resistance is associated with poor prognosis (21). Tumor response to radiation therapy involves a number of factors including tumor microenvironment and inherent (genetic) radiation susceptibility of cells within a tumor. Oxygen tension and pH have been shown to modulate cellular response to radiation, and changes in the tumor microenvironment can influence blood flow, which in turn changes these parameters. RR cells have been identified in primary cultures of head and neck tumors (22, 23). As the majority of solid tumors examined by molecular and cytogenetic techniques have evidence of genetic alterations, it is likely that radiation resistance arises as a result of those alterations.

Cellular irradiation produces a number of lesions within DNA, including single- and double-strand breaks. It is thought that dsb are the major lesion, and many studies of radiation response have focused on dsb repair (24). A study of human squamous cell carcinomas of the head and neck noted differences in the initial amount of damage (particularly dsb) induced in DNA between RR and relatively RS lines and differences in their ability to repair dsb (25). RS lines had more initial damage (measured by DNA neutral elution) and repaired damage more slowly than RR lines. DNA conformation was found to differ between the lines, suggesting that this may influence the initial amount of damage sustained and the ability of the cell to repair the damage (26). In addition to the variation in the amount of damage induced and the rate of damage repair, it has also been reported that RR cells spend longer in G2 than RS cells following irradiation (27, 28), suggesting that the response to radiation may involve alteration of cell cycle control genes.

In a previous report, we described recurrent chromosome changes consistent with a multistage model of tumorigenesis in a series of 10 cell lines derived from HNSCC (3). We have expanded that work to include an additional 6 lines and have identified karyotype changes that correlate with an in vitro response to radiation.

MATERIALS AND METHODS

Cell Lines. Cell lines were established according to methods published previously (29, 30). Table 1 summarizes details of the site of the tumor, Du, SF2, modal chromosome number, and ploidy of each line used in this analysis. Cell lines are listed according to their Du, from most sensitive to most resistant. Karyotypes of 10 lines used in this analysis have been reported previously (3). X-ray survival curves and cytogenetic harvest were undertaken on cells of the same passage whenever possible. If different passages were used, there was no more than 3 passages difference.

Cytogenetic Harvest and Analysis. Cells in an exponential growth phase were harvested according to methods published previously (3). Following G-banding using trypsin-Giemsa, 15 metaphase spreads were photographed and analyzed fully for each line. Metaphase spreads were selected for analysis based on technical quality.

In order to identify regions of chromosomal loss or gain in HNSCC lines, each line was assigned a ploidy number when the karyotype was completed. Ploidy was primarily based on the number of chromosomes in the modal cell population and was rounded to the nearest multiple of 23. However, the number of copies of each chromosome was also taken into account. For instance, a cell line with a count of 57 and 2 copies of each of 13 or more of the 22 autosome centromeres would be diploid. However, if there were three copies of most the chromosomes and one copy of a few chromosomes, the cell line would be triploid. This method of deriving the number of copies of a chromosome expected was used because it seemed more likely that aneuploidy arises as a result of aberrant mitotic separation, such as tripolar mitoses, than by simultaneous duplication of the majority of chromosomes within a cell by

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2 To whom requests for reprints should be addressed, at Cytogenetics Laboratory, New England Medical Center, 750 Washington Street, Box 188, Boston, MA 02111.
3 The abbreviations used are: HNSCC, head and neck squamous cell carcinoma; dsb, double-strand breaks; RR, radioresistant; RS, radiosensitive.
Table 1 Details of head and neck cell lines studied, including site of primary, in vitro D0, SF2, modal chromosomal number, passage, and assigned ploidy of line

<table>
<thead>
<tr>
<th>Line</th>
<th>Site of primary</th>
<th>D0 (Gy)</th>
<th>SF2[^a]</th>
<th>Modal[^c] no. (range)</th>
<th>Chromosome</th>
<th>Passage[^b]</th>
<th>Ploidy of line[^c]</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCC 61[^d]</td>
<td>Tongue</td>
<td>1.07</td>
<td>0.268</td>
<td>46 (45-46)</td>
<td></td>
<td>Mid</td>
<td>2N</td>
</tr>
<tr>
<td>SCC 25[^d]</td>
<td>Tongue</td>
<td>1.42</td>
<td>0.359</td>
<td>72 (59-78)</td>
<td></td>
<td>Late</td>
<td>4N</td>
</tr>
<tr>
<td>SQ 9G</td>
<td>Tonsil</td>
<td>1.46</td>
<td>0.350</td>
<td>52 (48-58)</td>
<td></td>
<td>late</td>
<td>2N</td>
</tr>
<tr>
<td>HNSCC 28</td>
<td>Larynx</td>
<td>1.54</td>
<td>0.391</td>
<td>69 (64-71)</td>
<td></td>
<td>Mid</td>
<td>3N</td>
</tr>
<tr>
<td>HNSCC 152</td>
<td>Parotid</td>
<td>1.54</td>
<td>0.537</td>
<td>73 (62-74)</td>
<td></td>
<td>Mid</td>
<td>3N</td>
</tr>
<tr>
<td>HNSCC 255</td>
<td>Parapharyngeal</td>
<td>1.56</td>
<td>0.579</td>
<td>49 (47-50)</td>
<td></td>
<td>Late</td>
<td>2N</td>
</tr>
<tr>
<td>HNSCC 167[^d]</td>
<td>Tonsil</td>
<td>1.81</td>
<td>0.650</td>
<td>70 (68-73)</td>
<td></td>
<td>early</td>
<td>3N</td>
</tr>
<tr>
<td>SCC 35[^d]</td>
<td>Pyriform sinus</td>
<td>1.85</td>
<td>0.469</td>
<td>65 (63-69)</td>
<td></td>
<td>Mid</td>
<td>3N</td>
</tr>
<tr>
<td>HNSCC 151[^d]</td>
<td>Tonsil</td>
<td>2.17</td>
<td>0.532</td>
<td>82 (75-85)</td>
<td></td>
<td>Early</td>
<td>4N</td>
</tr>
<tr>
<td>HNSCC 151[^d]</td>
<td>Tonsil</td>
<td>2.13</td>
<td>0.532</td>
<td>82 (75-85)</td>
<td></td>
<td>Early</td>
<td>4N</td>
</tr>
<tr>
<td>SQ 5[^f]</td>
<td>Pyriform sinus</td>
<td>2.26</td>
<td>0.509</td>
<td>85 (84-92)</td>
<td></td>
<td>Late</td>
<td>4N</td>
</tr>
<tr>
<td>HNSCC 135[^d]</td>
<td>Tonsil</td>
<td>2.27</td>
<td>0.572</td>
<td>77 (70-80)</td>
<td></td>
<td>early</td>
<td>3N</td>
</tr>
<tr>
<td>HNSCC 294[^d]</td>
<td>Tongue</td>
<td>2.27</td>
<td>0.563</td>
<td>80 (77-81)</td>
<td></td>
<td>Early</td>
<td>4N</td>
</tr>
<tr>
<td>SQ 208[^d]</td>
<td>Larynx</td>
<td>2.39</td>
<td>0.569</td>
<td>68 (64-69)</td>
<td></td>
<td>Late</td>
<td>3N</td>
</tr>
<tr>
<td>HNSCC 58</td>
<td>Tongue</td>
<td>2.56</td>
<td>0.400</td>
<td>55 (53-58)</td>
<td></td>
<td>Late</td>
<td>3N</td>
</tr>
<tr>
<td>JSQ 3[^d]</td>
<td>Nasal vestibule</td>
<td>2.63</td>
<td>0.674</td>
<td>57 (56-62)</td>
<td></td>
<td>late</td>
<td>2N</td>
</tr>
<tr>
<td>HNSCC 143</td>
<td>Oropharynx</td>
<td>3.33</td>
<td>0.727</td>
<td>70 (67-74)</td>
<td></td>
<td>mid</td>
<td>3N</td>
</tr>
</tbody>
</table>

[^a] Fraction of cells which survive 2 Gy.
[^b] Early, <7 passages; mid, 7–15 passages; late, ≥15 passages.
[^c] 2N, diploid; 3N, triploid; 4N, tetraploid.
[^d] Previously reported (3).

some other mechanism. Mitotic nondisjunction and tripolar mitoses have been described as characteristics of tumor cells (31).

Survival Curves. The D0 for each line was determined with X-ray survival curves as published previously (22). Briefly, exponential phase cells were irradiated with a GE MaxiMax X-ray generator at 250 kV peak and 26 mA, yielding a dose rate of 107 rads/min (1 rad = 1 cGy). The cells were grown for an additional 18–24 days after which they were fixed and stained with crystal violet. Colonies of >50 cells were scored as survivors. Dn, the inverse of the slope of the radiation survival curve, was calculated from the results of 2–4 experiments. Also, cell survival was assessed by a second measure, SF2, the fraction of cells surviving irradiation with 2 Gy.

RESULTS

As reported previously and illustrated in Figs. 1–4, the karyotypes of the cell lines were extremely complex (3). However, consistent rearrangements could be identified in all cells of each line, although no chromosomal rearrangements were seen in common among the lines. Intermetaphase variation was observed in some cell lines with additional marker chromosomes and/or losses or gains of one or two whole chromosomes in some but not all metaphases. There was no clear cosegregation of additional or missing chromosomes and/or marker chromosomes in any of the lines. The intraline variation was always less than the interline variation. The consensus karyotype of each line included all markers seen in at least three cells. As it was not possible to identify the components of all marker chromosomes in all lines with G-banding, there is underscoring of some breakpoints and some regions of deletion/duplication. There was no correlation between karyotype homogeneity in the cell lines and D0.

Eight lines were found to have D0 of <1.85 Gy (relatively RS) and 8 lines had D0 of >2.17 Gy (RR). Relative RR was defined as a £ > of greater than the upper limit of the range of D0 for normal fibro-
KARYOTYPE CHANGES CORRELATED WITH RADIosensitivity

Fig. 2. Representative karyotype of cell line HNSCC 28. Modal chromosome number, 69 (triploid). Karyotype interpreted as: 69, X, -sex, -sex, del(1)(q11), 2 X der(2)(2;15)(p13;q21.2), del(3)(q35), del(4)(q31.3), +der(4)(1;4)(q23;q21) 4pq+, i(5p), +i(5p). +del(6)(2;12)(q13;p11.2). der(7)(q7;7;q11.1). del(8)(pter-q24.1;hsr), del(9)(10;14)(p11.1;q24), del(11)(9;11.3)(11pter-q13. 3;9q11 ->q22;13q12->13qter). -13, der(13)(13;13)(p12;q13), der(13)(13;7)(p12;7), -14, i(14q), -15, -15, del(15)(1;15)(p22;p11.2), +16, -18, -22, 2 x i(22q), +2 small markers.

blasts, which is around 1.80 Gy. When the second measure of cell survival, \( SF_2 \), was used, the majority of lines remained within the same general grouping. The \( SF_2 \) of a normal keratinocyte line grown under the same conditions was found to be 0.424 (32). Five of 16 lines had \( SF_2 \) of <0.424, and 11 of 16 lines were relatively RR (\( SF_2 \) > normal keratinocytes).

In order to identify chromosome bands that may be the sites of known or unknown oncogenes and which may be activated in HNSCC as a result of chromosomal breakage and translocation, all identified breakpoints were plotted on an ideogram. Fig. 5 illustrates the results when the lines were grouped according to \( D_0 \). Each arrowhead represents one breakpoint occurring in one cell line, and multiple copies of the same rearrangement within a line were scored once.

It can be seen that the breakpoints were not distributed at random throughout the genome but instead tended to cluster, particularly in the pericentric regions of heterochromatin. Breaks were considered to cluster in a chromosome band if \( \geq 20\% \) of the breaks on a particular chromosome were in one band. For some chromosomes, however, the total number of breaks on the chromosome was sufficiently low that even one or two breaks constituted a cluster (e.g., chromosome 16). The distribution of breakpoints differed between RR and RS lines (grouped according to \( D_0 \)), although the total number of breaks was
Fig. 4. Representative karyotype of cell line HNSCC 151. Modal chromosome number, 82 (tetraploid). The karyotype was interpreted as: 82, XXXX, + der(X) (X::q22::p21).1, -12, 2 × del(3)(q25.1), -5, del(6)(q23), der(7)(7;13)(q14::p11.1), der(10)(10;13)(p11.1::q11.2), der(11)(11p1ter::q13.3::HSR), -12, psu dic(12)(p11.1::q11.2). In this cell, der(1) is with the 7's.

Fig. 5. Diagrammatic representation of the location of all identified breakpoints from 16 HNSCC cell lines. Breakpoints identified in RR lines are on the left of the chromosome, and those in RS lines are on the right. Each arrowhead represents one breakpoint. The boxes with arrows represent the formation of an isochromosome, with the arrow indicating which arm is duplicated. Multiple copies of the same marker chromosome in a line were scored once.

**DISCUSSION**

We and others have reported recurrent karyotype changes in cell lines and primary tumors derived from HNSCC. The most frequent changes were deletions of 18q, 10p, 3p, 6p, duplication of 7p, and clustering of breakpoints in 1p and 11q (3, 12, 33). Molecular studies on HNSCC cell lines and primary tumors have confirmed some of these cytogenetic observations: loss of sequences from the short arm of chromosome 3, between bands 3p14 and 3p25 (34); overexpression of the epidermal growth factor receptor, located on chromosome 7p (35–40); and amplification of a group of putative oncogenes on chromosome 11q13, HST1, BCL11, INT2, and PRAD1 (17–20). Other molecular alterations identified in HNSCC, including overexpression of similar in both groups (228 and 225 in RR and RS lines, respectively). Additionally, the frequency of isochromosome formation (indicated by the boxes with arrowheads) in RS lines was double that in RR lines (from 24 to 12). The major breakpoint clusters observed in RR lines were in 1p22, 1p13, and the centromeric regions of chromosomes 7, 13, and 14. For the RS lines, the major peak was in 11q13 and the centromeric regions of chromosomes 5, 13, and 14.

The distribution of breakpoints differed slightly when lines were grouped according to SF2, with the 5 lines with the lowest SF2 becoming the RS group (SF2 <0.424) and the remainder the RR group. Several apparent clusters became associated with the opposite phenotype, e.g., 1p13 cluster in D0 RR lines became a cluster in SF2 RS lines, suggesting no phenotype specificity. Several clusters remained associated with the same phenotype regardless of whether D0 or SF2 was used for sorting (1p22, 3p21, and 8p11.2 with RR). The cluster observed in 11q13 was lessened, but 5 breaks were observed in 5 lines, compared with 6 breaks in the 11 RR lines (data not shown).

In order to determine the sites of possible tumor suppressor genes which are most likely to be in regions of frequent deletion, a comparison was made of the number of copies of a region present in a line with the number expected from the ploidy designation, and any deviations were scored as deletions or duplications. For each line, loss or gain of a chromosomal region was recorded once, although in many cases multiple copies of the region were lost or gained. Overall, deletions appeared to be more frequent than duplications. Fig. 6 shows the occurrence of duplication and deletion in all the cell lines analyzed, listed according to D0 and SF2, from low to high in each case.

It can be seen that some deletions occurred in a majority of lines (≥ 10 of 16): deletion of 3p; 4p; 4q; 8p; 10p; 18p; 18q; and the short arm regions of the acrocentric chromosomes (13, 14, 15, 21, and 22). Two regions, 3q and 7p, were duplicated in the majority of lines. When the lines were sorted according to D0, it appeared that several chromosome changes were complementary between the RR and RS lines: 12p was duplicated in 3 of 8 RS lines and deleted in 5 of 8 RR lines; 10q was duplicated in 4 of 8 RS lines and deleted in 5 of 8 RR lines; distal 14q was duplicated in 3 of 8 RS lines and deleted in 5 of 8 lines. When the lines were arranged according to SF2, changes in distal 14q continued to segregate with phenotype, while those of 10q and 12p did not. Interestingly, 5 of 5 SF2 RS lines had 5q deletions, suggesting that this may be an additional region for investigation.
KARYOTYPE CHANGES CORRELATED WITH RADIOSensitivity

**A. Deletion, duplication, and deletion in the same arm**

**B. SP2 from graph insert**

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myc and ras oncogenes, have been reported (for review, see Ref. 41), as well as loss and/or mutation of the p53 gene (42–44).

Inherently RR cells have been identified in HNSCC and it has been suggested that these may account for therapy failure (22). Identification of any changes that mark such cells could aid in prediction of treatment outcome. When we compared the karyotypes of HNSCC cell lines that were RR with those that were RS in vitro, we found several karyotype changes which segregated with cell survival following irradiation. These included a cluster of breakpoints at 1p22 and deletion of distal 14q in RR lines; RS lines were characterized by more frequent breakpoints in 11q13 and duplications of distal 14q.

The regions of interest identified above contain several genes which might show altered expression in HNSCC. Genes assigned to distal 14q include FOS, a homolog of the FBJ murine osteosarcoma viral oncogene; TGFβ3, transforming growth factor β; ELK2, a member of the ETS oncogene family; and AKT1, homolog of a murine thymoma viral oncogene (45). At present, no candidate genes have been mapped to the breakpoint hotspot identified in RR lines, 1p22, which is an aphidicolin-inducible fragile site (46). Breakpoint clusters in 1p22 have been observed in yolk sac tumors (47) and the large cell diffuse lymphoma subgroup of non-Hodgkin's lymphomas (48). The RS breakpoint hotspot in 11q13 is the site of an amplicon that includes HST1, BCL1, INT2, and PRAD1, which has been demonstrated to have altered expression in HNSCC. The role, if any, of other genes remains to be determined.

Differences in response to radiation between cell lines are likely to be the result of alterations of many cellular systems, including DNA repair systems and possibly cell cycle control changes. The absolute number of genes involved in each of the many mammalian DNA repair pathways is presently unknown, but complementation experiments between different RS mutant cell lines suggest there are 8–9 complementation groups (49). To date, only one X-ray response gene has been cloned, XRCC1, and it has been localized to human chromosome 19q13.2 (50). The gene corrects a defect in single strand break joining in a hamster mutant cell line. Three other X-ray response genes have been mapped by complementation studies: XRCC2 to chromosome 7; XRCC4 to chromosome 5; and XRCC5 to chromosome 2 (51–53).

It has been suggested that chromatin structure differences may contribute to radiation response (26). Analysis of nucleoids in four of the lines studied showed differences between RR lines (JSQ-3; SQ20B) and RS lines (SCC25; SQ9G) consistent with alterations in supercoiling (26). Such changes may result in less efficient repair of damage in RS lines following irradiation as a result of altered or more relaxed DNA conformation. Additionally, alterations in chromatin structure may lead to centromeric replication errors and result in isochromosome formation, which are thought to be the result of misdivision of the centromere (54). The more frequent isochromosome formation observed in RS lines (24 compared with 12 in RR lines) may be a result of such modifications.

Subtle changes in cycle control could account for differences in response to radiation with sensitivity associated with a shortened period for repair. RS lines had frequent breaks in 11q13, the band to which PRAD1 has been assigned (18). PRAD1 is a G1 cyclin first identified in a subset of benign parathyroid tumors, which is also amplified in mammary tumors (18, 20). Overexpression of this gene may result in the failure of arrest in G1 following irradiation with concomitant failure of completion of DNA repair in RS lines.

In summary, we have shown that in addition to the recurring chromosomal changes described for HNSCC tumors, there are additional recurring changes that correlate with in vitro response to X-rays. The changes included deletion of 14q in one-third of RR cell lines and duplication of the same region in one-third of RS cell lines. Also, as RS cell lines frequently had breaks in chromosome 11 at band q13, a region frequently amplified in HNSCC, sensitivity may result from overexpression of PRAD1, a G1 cyclin, resulting in passage to mitosis before full repair of DNA damage with persistence of lethal DNA lesions. These observations suggest that radiation sensitivity would be the dominant phenotype in fusion experiments. We suggest that relative radiation sensitivity also may be associated with altered chromatin structure, which in turn may lead to an increased frequency of centromere replication errors.

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REFERENCES

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