Nonrandom Chromosome Losses in Stepwise Neoplastic Transformation in Vitro of Human Uroepithelial Cells

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Abstract

An in vitro/in vivo transformation system was used to study chromosome region losses in stepwise neoplastic transformation and progression of human uroepithelial cells. Complete cytogenetic analyses were done on 17 independent carcinomas derived using this system and showed that losses of chromosome regions on 3p (P = 0.0003), 6q (P = 0.01), and 18q (P = 0.0003) were nonrandom. The smallest common losses [i.e., 3(p13→pter), 6(q21→q23), and 18(q21.1→qter)] were in putative cancer suppressor gene regions. In addition, cumulative losses from a group of 10 chromosome arms (i.e., 1p, 1q, 3p, 5q, 6q, 9q, 11p, 13q, 17p, and 18q) frequently deleted in clinical carcinomas were very significant (P = 0.0005) compared to losses from all other arms. Loss of 3p and 18q both correlated with transformation to high grade carcinomas (P = 0.001 and P = 0.004, respectively). These data provide new evidence supporting hypotheses that chromosome regions 3(p13→pter) and 6(q21→q23) contain genes that suppress cancer development. These results also provide new data confirming the hypothesis that genetic loss(es) in the 18(q21.1→qter) region are associated with the development of high grade malignancies.

Introduction

Human cancers derived from multiple cell types show nonrandom chromosome losses (1) and genetic deletions (2). For example, direct examinations of clinical cancers using cytogenetic and/or molecular genetic techniques show deletions on 1p in neuroblastomas (3); 1q in breast cancers (4); 3p, 11p, 13q, and 17p in lung cancers (5); 3p in renal cell carcinomas (6); 5q, 17p, and 18q in colorectal carcinomas (7); 6q, 11p, and 17p in ovarian cancers (8); and 6q, 9q, 11p, and 17p in bladder carcinomas (9-13). These observations have led to the hypothesis that losses of genes on these chromosome arms lead to development of cancer (2). We have used a multistep in vitro/in vivo HUC transformation system to test this hypothesis. We present here the first example of nonrandom deletions of putative cancer suppressor gene regions in association with stepwise transformation in vitro of any human epithelial cell type.

Materials and Methods

Detailed cytogenetic analyses were performed on 17 independent uroepithelial carcinomas (T-HUC carcinomas) obtained using our multistep HUC transformation system (Fig. 1A). A nontumorigenic, SV40-immortalized clonal cell line (SV-HUC) was established after infection of HUC with SV40 (14). SV-HUC cells have a pseudodiploid karyotype with no cytogenetically apparent significant net losses or gains of chromosome regions (14). SV-HUC were exposed in vitro to 3-methylcholanthrene (15) or ABP or its more reactive metabolites (16) or transfected with mutant EJ/ras (17). 3-Methylcholanthrene was chosen because it is a representative potent polycyclic hydrocarbon carcinogen, and ABP was used because it is an important human chemical carcinogen that is present in cigarette smoke and increases the risk for bladder cancer (18). The mutant EJ/ras was used because it was derived from a human bladder cancer cell line. Treated SV-HUC were inoculated into athymic nude mice to test for neoplastic transformation as described previously (15, 17). Control SV-HUC were inoculated and did not produce tumors.

The 10 initial neoplasms generated showed different carcinoma phenotypes reminiscent of the broad spectrum of biological phenotypes seen in clinical bladder cancers (18). These included low and high grade transitional cell carcinomas and squamous cell carcinoma and also undifferentiated cancers. T-HUC carcinomas also differed in their in vivo growth behavior. Some cancers grew in an indolent manner, while others were classified as progressive or aggressive. Cell lines were established from the T-HUC carcinomas and on reinoculation most produced cancers that were similar to the initial neoplasms. However, a few tumor cell lines spontaneously progressed after 2-4 passages in vitro (16) and formed progressed, more aggressive, or higher grade tumors on reinoculation. One cell line, MC-T11, that was derived from a Grade 1 neoplasm and did not spontaneously progress was exposed again in vitro to chemical carcinogens and then formed progressed tumors on reinoculation (Fig. 1A). Fig. 1A shows the derivation of the 10 initial and 7 “progressed” higher grade T-HUC carcinomas used in this study.

Results

Cytogenetic analyses of these 17 T-HUC carcinomas showed that all were clonal and derived from the parental SV-HUC. Chromosome duplications were infrequent (the subject of a separate study). In contrast, chromosome arm losses occurred frequently in association with both the 10 tumorigenic transformation and the 7 neoplastic progression events studied, averaging 6.9 losses per event. Overall, 18% of chromosome arms evaluated in T-HUC carcinomas showed losses, which is comparable to 20% of evaluable chromosome arms in colorectal cancers that showed allelic deletions (19).

A chromogram [similar to an allelotype (19)] of chromosome arms lost in association with tumorigenic transformation or neoplastic progression of SV-HUC was constructed [Fig. 2]. Statistical analysis (20) showed significant nonrandom overall losses of 3p (P = 0.0003), 5p (P = 0.01), 6q (P = 0.01), and 18q (P = 0.0003) in T-HUC carcinomas. Examining only losses associated with the 10 initial transformation events and not considering carcinoma phenotype, 5p and 3p losses were very significant (P = 0.0001 and P = 0.007, respectively), but 6q...
Fig. 1. A, generation of 17 T-HUC carcinomas using the HUC in vitro/in vivo transformation system. To generate the 10 initial cancers (T) and the 7 higher grade progressed cancers (pT and ppT) used in this study, SV-HUC (13) were exposed in vitro to 3-methylcholanthrene (MCA) (15), ABP, or its reactive metabolites N-hydroxy-4-aminobiphenyl (HABP) or N-hydroxy-4-acetylaminobiphenyl (HAAABP) (16) or transfected with EJ/ras (17). Cells were then inoculated into athymic nude mice (HSD/Athymic Nude-mu) using 2-5 × 10⁶ cells/site to test for tumorigenic transformation. The growth kinetics of neoplasms was monitored by weekly measurements. All tumors were classified based on their growth kinetics as aggressive (>0.5 cm at 10 weeks), progressive (persistent growth, but <0.5 cm at 10 weeks), or indolent (small nonregressing nodules). Tumors were removed before they reached 1.0 cm and tissue was fixed for pathology and used to initiate tumor cell cultures (14–16). Tumor cell lines were reinoculated to test for progression. Controls did not produce tumors. B, chromosome arm losses in stepwise transformation in vitro of a single T-HUC carcinoma. Shown here is a specific example of significant chromosomal losses associated with each step in the evolution of a high grade clonal cancer using the in vitro/in vivo transformation system. * loss of one chromosome arm in this tetraploid cancer.

15q and 18q losses were also significant (all, P = 0.04).

Approximate unconditional χ² analyses associating losses with cancer phenotypes showed a strong correlation between 3p loss and undifferentiated or poorly differentiated Grade III carcinomas (P = 0.001). Loss of 18q was highly correlated with progression of Grade I or II noninvasive cancers to invasive Grade III cancers (P = 0.004), whereas 6q and 3p losses both correlated with aggressive tumor growth kinetics (both, P = 0.01). Losses of 5p and 15q, although nonrandom, did not associate with transformation to high grade cancers (P = 0.12 and P = 0.21, respectively), but these may be important in early steps of HUC transformation.

We noticed losses in T-HUC carcinomas from many chromosome arms frequently deleted in clinical cancers (3–13) and therefore investigated the overall significance of the cumulative losses of 10 such arms (i.e., 1p, 1q, 3p, 5q, 6q, 9q, 11p, 13q, 17p, and 18q). Losses from this group of chromosome arms were highly significant (P = 0.0005) compared to losses from all other arms. Significantly, each T-HUC carcinoma evolved in a unique and independent manner: e.g., no two cancers in this series showed exactly the same chromosome losses. A specific example of chromosome arm losses associated with the evolution of one clonal cancer in this series from low to higher grades is shown in Fig. 1B.

Some but not all T-HUC carcinomas showed large chromosome arm deletions. Notably, the least common regions deleted on 3p, 6q, and 18q, i.e., 3p(13→pter) (21), 6q(21→q23) (Fig. 3A), and 18q(21.1→pter) (Fig. 3B) were precisely in putative cancer suppressor gene regions for renal cell (6), ovarian (8), and colorectal (22) carcinomas, respectively. Importantly, allelic deletion analyses in T-HUC carcinomas using probes mapped to these regions have confirmed all the 3p (21) and the 18q deletions, and studies to confirm 6q losses are in progress. Thus, cytogenetic analysis was accurate in identification of the smallest common chromosome regions deleted and useful as a prelude to molecular analyses.

Fig. 2. Chromogram of chromosome arm losses. Shown here are all the chromosome arm losses (a total of 117) that accompanied both the 10 initial transformation events (A) and the 7 neoplastic progression events (B) shown in Fig. 1A. Full box, one loss from a near diploid cancer or 2 losses from a near tetraploid cancer; half-boxes, 1 loss from a tetraploid cancer. All the short (p) and long (q) arms of autosomal chromosomes were scored excepting the satellites arms 13p, 14p, 15p, 21p, and 22p. Chromosome arm losses associated with the initial transformation events were not scored again when the carcinomas progressed. Probabilities associated with the number of chromosome arm losses were computed from a binomial model under the null hypothesis that loss events were distributed randomly. Separate analyses were performed for chromosomes with satellites under the assumption that these were not likely to be lost independently. *Arrows*, significant overall chromosome arm losses (P < 0.05). Chromosome arm losses were based on detailed cytogenetic analysis of 10–20 metaphases of each tumor using Giemsa-banded chromosomes (14). Tumor cell cultures were analyzed between passages 2 and 4. A chromosome arm loss was counted if >75% cells analyzed showed the loss.

* Unpublished observations.
Discussion

Cytogenetic analysis has been an invaluable tool in the initial identification of genetic changes associated with the development of cancer (1). For example, karyotypic analyses of human bladder cancers show cytogenetically visible losses on 6q (20%), 9q (24%), and 11p (40%) (1, 9, 10). Allelic deletion analyses subsequently confirmed the loss of genes on 9q (67%) and 11p (40%) and unmasked 17p losses (63%) (11-13).

The HUC transformed in vitro and analyzed in this study showed losses involving 3p, 6q, and 18q. Losses on 11p were also observed in our system. Although few losses of 9q were seen, every T-HUC had a translocation involving chromosome 18q (18). The fact that T-HUC did not show frequent cytogenetic losses of 17p, consistent with cytogenetic studies of clinical bladder cancers (1, 9, 10). Allelic analysis of 17p genes in T-HUC carcinomas is in progress.

In our study, cytogenetically visible losses of 3p and 18q occurred more frequently than in clinical bladder cancers. Although cytogenetic losses of 3p have been observed in bladder cancers, 3p duplications and t3(p14) are more common (9, 10). We associate losses of 3p primarily with high grade poorly differentiated uroepithelial cancers and show that the smallest common deletion is in the 3(p13—pter) region (21). Thus, our results predict a suppressor gene for high grade uroepithelial cancers in this region. Since the majority of bladder cancers are superficial and/or low grade transitional cell carcinomas (18), our results are not necessarily inconsistent with cytogenetic studies of clinical bladder cancers. In support of this view, loss of 3p has been associated with high grade renal cell carcinoma but is not found in papillary tumors (23).

Loss of 18q has been associated by Fearon and Vogelstein (2) with the later stages of colorectal cancer. In addition, in a report of an unusual high grade invasive bladder cancer in an adolescent male, one of only two changes in an otherwise diploid karyotype was a translocation involving chromosomes 18q and 17p (24). Furthermore, a significant association has been observed between loss of heterozygosity for 18q and 17q genes and aggressive human breast cancers (25). Thus, our observation that loss of genes in the 18(q21.1—qter) region is correlated with progression to high grade uroepithelial carcinoma is consistent with results obtained using clinical cancers and is the third example to our knowledge associating 18q loss with carcinoma neoplastic progression.

The studies reported here are complementary to studies done using clinical samples of carcinomas of different grades and stages. We have studied chromosome losses associated with each step of clonal evolution of individual neoplasms from low grade to higher grades. Such studies are not readily done using clinical cancers. In contrast to clinical cancers, the transforming agents in this in vitro/in vivo system are known. Some of these cancers were derived after exposure to ABP, a significant, relevant, and ubiquitous human carcinogen for bladder (18) and possibly colonic epithelium (26). It will be important and possible using this system to determine if the remaining alleles in suppressor genes showing allelic losses are mutated. This in vitro/in vivo transformation system will also be useful for experimental studies using chromosome or gene transfer techniques of the biological significance of suppressor gene losses (or oncogene activation) at each step of tumorigenesis. Finally, this system can be used to study the putative modulating effects of the SV40 T-antigen on the significant requirement (12, 13) for loss of the p53 17p or 13q RB putative suppressor genes in human uroepithelial cell tumorigenesis.

In summary, we have reported here the first example of nonrandom genetic losses associated with tumorigenic transformation and neoplastic progression in vitro of any human epithelial cell type. Although the tumors generated in this in vitro system all derived from one clonal parental cell line, these showed heterogeneous carcinoma phenotypes. It was possible to associate specific chromosome losses with certain cancer phenotypes. Because the chromosomal regions lost contain putative cancer suppressor genes, this system is potentially valuable for in vitro studies to determine the significance of these losses in tumorigenesis.

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References

LOSS OF 3p, 6q, AND 18q IN EPITHELIAL TRANSFORMATION


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