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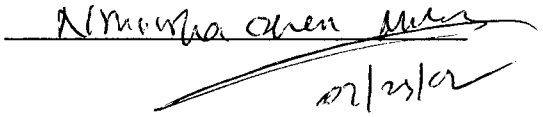

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13. ABSTRACT ***(Maximum 200 Words)***

The purpose and scope of this project was to utilize a functional approach for the physical mapping and identification of a novel tumor suppressor gene for prostate cancer within chromosome 10p. The major findings include the development of a technology for serial microcell fusion to transfer defined 10p fragments into a mouse A9 fibrosarcoma cell line. Once characterized by FISH and microsatellite analyses, the 10p fragments were subsequently transferred into PC-3H to generate a panel of microcell hybrid clones containing overlapping deletions of chromosome 10p. *In vivo* and microsatellite analyses of these PC hybrids identified a small chromosome 10p fragment (an estimated 31 Mb in size inclusive of the centromere) that when transferred into the PC-3H background, resulted in significant tumor suppression and limited a region of functional tumor suppressor activity to chromosome 10p12.31-q11. This region coincides with a region of LOH demonstrated in prostate cancer. These studies demonstrate the utility of this approach as a powerful tool to limit regions of functional tumor suppressor activity. Furthermore, these data used in conjunction with data generated by the Human Genome Project lent a focused approach to identify candidate tumor suppressor genes involved in prostate cancer.

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INTRODUCTION

The purpose and scope of this project was to utilize a functional approach for the physical mapping and identification of a novel tumor suppressor gene for prostate cancer within chromosome 10p. The major findings include the development of a technology for serial microcell fusion to transfer defined 10p fragments into a mouse A9 fibrosarcoma cell line. Once characterized by FISH and microsatellite analyses, the 10p fragments were subsequently transferred into PC-3H to generate a panel of microcell hybrid clones containing overlapping deletions of chromosome 10p. *In vivo* and microsatellite analyses of these PC hybrids identified a small chromosome 10p fragment (an estimated 31 Mb in size inclusive of the centromere) that when transferred into the PC-3H background, resulted in significant tumor suppression and limited a region of functional tumor suppressor activity to chromosome 10p12.31-q11. This region coincides with a region of LOH demonstrated in prostate cancer. These studies demonstrate the utility of this approach as a powerful tool to limit regions of functional tumor suppressor activity. Furthermore, these data used in conjunction with data generated by the Human Genome Project lent a focused approach to identify all genes and ESTs in this region which can now be screened on microarrays to identify candidates for PAC-1.

A. BODY

Task 1-PCR subtractive hybridization initiated using characterized hybrids from microcell hybrid panel (suppressed hybrid against unsuppressed hybrid). Candidate partial cDNAs identified, screened for differential expression in hybrids and parental cells and sequenced (months 1-10).

Task 2-Narrowing of the region containing PAC-1 functionally using defined fragment-containing microcell hybrid clones constructed by the transfer of 10p deletion chromosomes into PC-3H. Positional cloning efforts initiated using STSs and ESTs from the nonoverlap region between suppressed and unsuppressed hybrid clones to screen PAC/BAC libraries. Clones fingerprinted to construct contig (months 1-20).

Task 3-Mapping to slot blots of P1/PAC/BAC clones used to construct a contig across the region of nonoverlap (months 3-20).

Task 4-cDNAs that map into the nonoverlap region and that are differentially expressed in prostate tumor/normal samples as well as hybrid lines and prostate cell lines used to screen cDNA libraries to isolate full length cDNAs for PAC-1 (months 15-30).

Task 5-Tissue distribution of candidate cDNAs determined by Northern analysis (months 25-30).

I. <u>Progress Related to Tasks Over Grant Period</u>

Our previous functional data provided a useful correlative with cytogenetic and LOH analyses that implicate chromosome 10p in prostate cancer. An obstacle to isolating PAC-1 at this stage, however, was the sheer size of the introduced region. 10pter-q11 is an estimated 55 Mb in size, still too large to practically clone any one gene that is responsible for tumor suppression in our model. To promote the feasibility of isolating PAC-1, subsequent studies focused upon limiting the

region that could contain PAC-1 and generating reagents for this gene's isolation. Based on the previous functional data, we were interested in determining whether a region of hemizygosity or a homozygous deletion within the region 10pter-q11 could be detected in our recipient prostate cancer cell line PC-3H. Identification of one or more such regions would implicate them as genomic regions harboring candidates for tumor suppressor loci. Equally important is the demonstration of functional genetic complementation of these candidate regions. To this end, we have focused upon generating smaller, defined fragments of the region 10pter-q11 in order to functionally limit the tumor suppressor locus containing PAC-1. Thus, in a concerted molecular and somatic cell genetic approach, we have generated important information that physically and functionally maps PAC-1 within 10pter-q11, as well as generated reagents that will figure prominently in the isolation of this important gene.

II. Limiting the Regions Within Chromosome 10p Containing PAC-1

Task 1 involved an expression-based strategy to identify candidate cDNAs for *PAC-1* from the 10p interval.
Task 2 superceded this task. Progress in physical mapping of the *PAC-1* region in addition to rapid progress in the Human Genome effort to generate BAC contigs and initiate sequencing of the region resulted in the concentration of effort on task 2. Task 2 involved the limiting of the *PAC-1* critical region using Comparative Genomic Hybridization (CGH) analysis in combination with microsatellite analyses on PC-3H to determiine if a region of hemizyosity or homozygous deletion could be identified which could narrow the region containing the tumor suppressor locus PAC-1. Secondly, defined microcell hybrid deletion clones were generated for the physical mapping of the *PAC-1* critical region.

A. Comparative Genomic Hybridization Analysis of PC-3H

Results from CGH analysis indicated gains of chromosomes 8q21.1-qter (4/5 metaphases), 11p15 (5/6), and 17q21-qter (5/6). No gains were identified on chromosomes 2, 4, 9, and 15. Results also indicated a gain of chromosome 19 in its entirety and also gains involving the entire short arm of chromosome 18 and the entire long arm of chromosome 14. Losses were identified in the chromosomal region 8pter-q13 in a high proportion (4/5 metaphases). No losses were found on chromosomes 3, 11, and 19. Losses involving the entire arm of a chromosome included 8p. Chromosome 9 appeared to be completely lost.

Previous reports describe the absence of intact chromosome 10 as a consistent feature in PC-3. Our laboratory's initial cytogenetic and chromosome painting analysis of our subline PC-3H supports their findings, demonstrating that fragments of chromosome 10 were scattered throughout the genome of PC-3H. CGH analysis indicated six of seven analyzed metaphase spreads with a region of loss between 10p12 to 10p15 (summarized in Table I). The smallest region (identified in one cell) was from 10p13-p14. Two cells showed the lost region encompassing 10p12-p14. A single cell demonstrated the loss between 10p13-p15. Finally, two cells showed the largest region of loss, spanning 10p12-p15. The approximate size of the genomic region containing losses thus ranged from 6 Mb to 30 Mb. In addition, PC-3H displayed an amplification of the region 10p12-q23. The region of amplification extended to the proximal portion of 10p13 in one cell (out of seven analyzed metaphases), and to 10p12 in three additional cells. The remaining three cells showed amplification extending to 10p11. On the long arm of chromosome 10, the amplification endpoints

ranged from 10q22 (in three cells) to 10q23 (in four cells). Two of seven cells also showed gains of the 10p15 region. Taking this data into account, these results implied a common region of loss on the short arm of chromosome 10 extending from 10p13-10p14. However, the margin of error in determining the boundaries of the deletion can be 3-5 Mb, meaning that the region of hemizygosity could be larger than that defined by 10p13-p14. Also, a majority of analyzed metaphase spreads show the proximal boundary within 10p12 and the distal boundary within 10p14-p15. Therefore, the common region of loss could extend from within 10p12 to within 10p15.

To determine the status of the short arm of chromosome 10 in PC-3H, chromosome painting was performed using the subchromosomal region 10pter-q11 as a probe onto PC-3H metaphase spreads. The analysis indicated that no independent, intact copies of the subchromosomal region were apparent within the PC-3H genome. Rather, fragments of chromosome 10p had integrated within several chromosomes. These results agreed with previous reports that record the absence of chromosome 10 in PC-3 and the rearrangement of this chromosome within other marker chromosomes. Furthermore, these results implied that losses and gains of chromosome 10p material might have occurred either as a result of rearrangement, or perhaps as a condition for it.

These results, taken in conjunction with previous cytogenetic analyses of PC-3 and our subline PC-3H, suggest that along with the rearrangement of chromosome 10 within PC-3H, deletion and amplification of subchromosomal regions of chromosome 10 occurred. Furthermore, these data suggest a candidate tumor suppressor locus (or loci) within 10p12-p15, a region of approximately 30 Mb.

B. Microsatellite Analysis of PC-3H

In addition to CGH analysis, our laboratory performed microsatellite analysis using thirty-six polymorphic markers that map to chromosome 10p12.1-p15.3 to identify regions of hemizygosity or homozygous deletion. The markers used for this study each exhibited a degree of heterozygosity of 70% or greater. As shown in Table II, all of the markers demonstrated homozygosity in PC-3H. These results suggest that PC-3H may be hemizygous for all or part of the short arm of chromosome 10.

This analysis also identified a single marker within 10p15.3, D10S1145, that demonstrated complete absence of the estimated 250-400bp PCR product. To rule out the possibility of PCR failure, multiplex PCR was performed using D10S1716 in addition to D10S1145, with the result indicating that the reaction was capable of amplifying D10S1716 product from PC-3H DNA with the concomitant absence of D10S1145 product. These results suggested that PC-3H was homozygously deleted for this subregion within 10p15.3.

To further limit the deletion in PC-3H using PCR, primers were designed that were specific for regions upstream and downstream of the homozygously deleted locus using available genomic sequence (Fig. 1). In addition, a panel of prostate cancer cell line DNA samples were screened to determine the status of this locus and the flanking regions. All prostate cancer cell lines were positive for the locus D10S1145 and its flanking regions. D10S1145 primers originally demonstrated homozygous deletion in PC-3H); however, PC-3H was positive for PCR products corresponding to upstream and downstream flanking sequences. To illustrate the deletion by demonstrating a shift in product size, newly designed primers were used to PCR amplify across the region. PC-3H generated a PCR product of the expected size range for this region. These results suggested that PC-3H had not suffered a homozygous deletion at this locus, but a second possibility was that the primers designed were not specific for the region of interest.

To confirm the specificity of the primer sets used to limit the putative homozygous deletion at 10p15.3 within the prostatic carcinoma cell line PC-3H, PCR products were generated from regions immediately upstream, downstream and spanning the locus D10S1145 from genomic DNA samples and the DNA sequence of these products determined. Sequence analysis showed that PCR products generated from all DNA samples were specific for regions upstream, downstream and spanning the locus D10S1145. Therefore these primer sets were indeed specific for the 10p15.3 locus, and PC-3H was positive for this locus. However, PC-3H showed deviations in sequence when compared to all other samples. As shown in Figure 2 (in pink), PC-3H demonstrated four single base changes within a 160-bp region located in an *Alu* repetitive element that is 90% identical to the *Alu*-Sx subfamily (Jurka and Milosavljevic, 1991). One of the base changes occurred within the sense primer annealing site for D10S1145, causing a mismatch between the primer sequence and the genomic sequence in PC-3H. None of the four base changes were observed in three normal male controls, MDAPC2b (a newly established prostate carcinoma cell line described by Navone et al (1997)), and HA(10p)A (an A9 somatic cell hybrid containing an introduced human chromosome 10pter-q11).

To test the hypothesis that the original PCR result of a null allele was due to a mismatch between the sense primer and PC-3H genomic sequence, a new sense primer was designed to exactly match the PC-3H genomic sequence. PCR using this new primer generated a product within the expected range for this marker, suggesting that the base change in PC-3H within the D10S1145 sense primer annealing site was responsible for the initial result of homozygous deletion. However, there is a possibility that this band is the result of nonspecific priming of the antisense primer. Comparison of the product size derived from PC-3H with the range of nonspecific products generated by using the antisense primer alone shows that the PC-3H product falls within this range of products.

Alu elements have been shown to act as enhancers and repressors of gene expression (Britten, 1996). Based on those findings, the base changes within the region of interest could represent genomic alterations in PC-3H that alter gene expression. A second possibility was that these changes simply defined this region in PC-3H as a better match to an *Alu* subfamily member (*Alu*-Sx, Jurka and Milosavljevic, 1991) than those found in the human controls and the prostate cancer cell line MDAPCa-2b. To distinguish between these two possibilities, enhancer searches using NSITE and TRANSFAC algorithms/databases were performed, and a BLASTN search compared the affected region in PC-3H with the wild-type sequence against the database of select *Alu* repeats (REPBASE). The results of the enhancer searches yielded many (645 total, data not shown) high-scoring matches to enhancer consensus sequences, suggesting the putative role of this region to act as a regulator of gene expression. However, the results of the BLASTN search showed that the affected region in PC-3H was indeed a better match for *Alu*-Sx, as well as *Alu*–Sq (Jurka and Milosavljevic, 1991), subfamilies than the sequence found three normal male controls and two cell lines, with the four single-base changes being responsible for the higher scores generated by PC-3H sequence. Therefore, there was no homozygous deletion at D10S1145 in PC-3H, but a hemizygous sequence change in the *Alu* element upstream of this locus.

To address the possibility that these base changes represented normal variations within this region, DNA sequence comprising the *Alu* element and upstream flanking sequence were analyzed using the BLAST algorithm to search the single-nucleotide polymorphism (SNP) database (dbSNP). The Doubletwist genome analysis program was also used to map any SNPs that might fall within this region. The results of both approaches did not identify any known SNPs within this region.

Thus, the significance of specific base changes in PC-3H within this *Alu* element that are not found in three normal male controls and two cell lines remains unclear.

If this result is of significance, then one could hypothesize that mutations of this *Alu* element might result in the inappropriate gene expression of flanking genes. A BLAST search of 87.5 kb of genomic sequence containing this region identifies at least two candidate gene. One is 31kb downstream from the affected region and is made up of five expressed sequence tags (ESTs) clustered at the poly-A tail of an unknown gene. Reverse-transcription-PCR (RT-PCR) experiments to isolate larger cDNA segments for this gene have been unsuccessful. The other gene is 1.5kb upstream from the affected region and comprises greater than 300 ESTs that are part of a UniGene cluster for the partial cDNA KIAA0217 whose function is unknown, but the partial protein sequence of this gene contains an RNA binding domain. The 5' end of this cDNA sequence was extended an additional 400 bases, using available ESTs and bacterial artificial clones (BACs), and a significant homology of this novel sequence to a domain of a cell adhesion molecule was observed. In addition, an alternatively spliced exon was identified in a subset of ESTs belonging to this gene. This candidate gene now spans two BAC clones. The Doubletwist genome analysis tool confirmed these findings, and a comparison with the Human Genome Project Working Draft at the University of California at Santa Cruz (UCSC) also showed the same result for this genomic region, with the added finding that Affymetrix, Inc. had extended KIAA0217 even further using gene prediction algorithms.

To determine whether either of these genes were potential candidates for a tumor suppressor gene involved in prostate cancer, preliminary RT-PCR analyses of both candidate genes was performed. Both genes were expressed in PC-3H by RT-PCR, suggesting that they did not play a role in tumor suppression. Northern analysis using a probe specific for the candidate gene KIAA0217 whose end lies 1.5 kb upstream of the locus D10S1145 showed expression of the estimated 7 kb transcript in both tumorigenic PC-3H and the nontumorigenic PC hybrid containing the introduced 10pter-q11. Expression levels in the suppressed hybrid PC(10p)D did not vary significantly from PC-3H, suggesting that KIAA0217 is not affected by mutations in the *Alu* element.

These data indicate that in addition to the finding of implied hemizygosity for the short arm of chromosome 10 in PC-3H, we have identified a region at 10p15.3 that displays four single-base changes that distinguish this tumorigenic prostate cancer cell line from three normal male control DNA samples, as well as from the prostate cancer cell line MDAPCa-2b and an A9 somatic cell hybrid containing human 10pter-q11. The significance of these changes are yet to be determined.

III. Functionally Mapping PAC-1 Using Serial Microcell Fusion

Previously, our laboratory identified a novel tumor suppressor locus involved in prostate cancer within the region 10pter-q11 (Sanchez *et al*, 1996). Using microcell fusion, we introduced a single human chromosome 10 dominantly tagged with the selectable marker *neo* into a subcloned cell line of PC-3 (denoted PC-3H), which demonstrates an absence of intact chromosome 10. PC hybrids containing the introduced intact chromosome 10 demonstrated dramatic tumor suppression *in vivo* when compared to the parental line PC-3H. Importantly, PC hybrids containing only the introduced subchromosomal region 10pter-q11 were equivalent in their ability to suppress tumorigenesis as exhibited by PC hybrids containing intact chromosome 10.

Subsequent functional assays to localize prostate tumor suppressor loci within 10pter-q11 required the generation of donor hybrid cell lines containing defined, overlapping subchromosomal fragments of this region. Other laboratories have modified the technique of microcell fusion to generate small subchromosomal fragments by irradiation of microcells prior to fusion to rodent recipient cells (Dowdy *et al*, 1990). While this technique has proven useful for the physical mapping of the human genome (Goss and Harris, 1975), as well as for the generation of molecular probes and the positional cloning of genes (Fountain *et al*, 1989; Jones *et al*, 1991; Leach *et al*, 1993), the use of fragments produced by this method could result in the generation of false negatives in a functional assay for tumor suppression. Radiation-induced mutations within a tumor suppressor locus could inactivate the gene of interest, giving rise to *in vivo* tumor formation from a hybrid that is genotypically relevant.

Another approach we have used to generate subchromosomal fragments takes advantage of an interspecific cross to segregate segments of the foreign chromosome (Killary *et al*, 1992). By using a human chromosome 3 dominantly tagged with the selectable marker *neo*, introduced this chromosome into mouse A9 fibrosarcoma cells. We next subcloned two resultant hybrids initially in the absence of the antibiotic G418 to encourage segregation of the entire introduced chromosome or the region of the chromosome containing the *neo* gene. Five subclones, upon reapplication of G418, demonstrated sensitivity to the antibiotic yet exhibited dramatic tumor suppression *in vivo*. Molecular analysis of these subclones revealed the presence of the human subchromosomal region 3p21-p22 incorporated within a murine chromosome in the mouse A9 background. The *in vivo* tumor suppression was thus attributed to this subchromosomal region that was stably maintained by integration within the recipient genome.

In a typical microcell fusion experiment, the introduction of a single, intact normal chromosome results not only in the generation of hybrids containing the intact chromosome, but also hybrids that contain fragments of the introduced chromosome (Fournier and Frelinger, 1982; Fournier and Moran, 1983; Leach *et al*, 1989). The most common fragments generated are the result of simple terminal deletion. Based on this observation, we developed a technique, termed serial microcell fusion, to generate defined subchromosomal fragments of the region 10pter-q11. Furthermore, fragments generated by this method localized a functional tumor suppressor locus involved in prostate cancer to 10p12.31-q11 (approximately 31 Mb).

As a first step in serial microcell fusion, the microcell hybrid HA(10p)A, which contains the human subchromosomal region 10pter-q11 in the mouse A9 cell background, was serially transferred into recipient mouse A9 cells. The resulting hybrids from this interspecific cross could then be rapidly screened by fluorescent *in situ* hybridization (FISH) and microsatellite analysis to determine the boundaries of the introduced fragment (for a schematic of serial microcell fusion, see Fig. 3). Serial microcell fusion generated thirty-six HA2(10p) hybrid clones. Examination of these clones using microsatellite analysis identified eighteen clones that demonstrated a deletion of at least one of the seventeen microsatellites analyzed for chromosome 10p. All clones appeared to retain the most distal marker on 10p. Thus the hybrid clones generated in this experiment seemed to represent defined interstitial deletions of 10p.

Our initial analysis of the donor line HA(10p)A indicated that the integration site for the dominant selectable marker gene *neo* was located near the centromere on the q arm (Sanchez *et al*, 1996). HA(10p)A was a hybrid clone generated from a microcell fusion experiment using a pool of donor human foreskin fibroblast clones (each containing a different human chromosome tagged with *neo*) and the recipient mouse A9 cell line. This fusion had generated another hybrid clone, HA(10)A, that contained an intact chromosome 10 containing the *neo* gene integrated within 10p15,

as shown in a FISH analysis of this clone using the *neo* gene as a probe. Our laboratory hypothesized that two *neo* genes were integrated within the 10pter-q11 fragment in HA(10p)A. One gene was integrated on the q arm near the centromere, as previously reported, and the second gene would be integrated near 10pter. Thus we had used this doubly-tagged subchromosomal fragment to generate interstitial deletions of 10p using serial microcell fusion. FISH experiments using the *neo* gene as a probe onto HA(10p)A metaphase chromosomes confirmed this hypothesis, and also showed that a subpopulation of HA(10p)A cells contained both a double *neo*-tagged fragment, as well as a single *neo*-tagged fragment, with *neo* integrated at 10q11. Therefore the location of *neo* at both distal ends of the fragment forced the retention of these ends and the generation of interstitial deletions of 10p. However, it was also possible that a subpopulation of HA2(10p) clones would have received the single *neo*-tagged fragment. FISH analysis using *neo* as a probe onto a subset of HA2(10p) clones demonstrated not only the retention of either a singly- or a doubly-tagged fragment, but the fact that the introduced fragment was autonomous and visibly smaller that the original 10pter-q11 fragment used for the serial microcell fusion experiment. Thus, serial microcell fusion had generated smaller, defined fragments of 10pter-q11.

A panel of five HA2(10p) clones represented defined, overlapping interstitial deletions of chromosome 10p (Figure 4). Two clones (HA2(10p)19 and HA2(10p)33) appeared rearranged for one marker, and were excluded from further study. Of the remaining three, HA2(10p)11 contained the largest deletion, from 10p12.31-p15.3. This deletion is an estimated 20 Mb in length. HA2(10p)27 had a deletion from 10p12.1-p13, corresponding to an approximate size of 14 Mb. Upon reexamination of HA2(10p)2, which appeared to have a small deletion at 10p14 (~6-7 Mb), a false negative result for the marker D10S189 determined that this clone was intact for this region. HA2(10p)2 was thus excluded from further analysis.

To functionally dissect tumor suppressor loci within the short arm of chromosome 10, HA2(10p)11 was used as the donor line for microcell fusion into PC-3H. HA2(10p)11 demonstrated, by FISH analysis, a significant fraction (55%) of cells containing two autonomous fragments, each singly-tagged with *neo*. It is unknown if this *neo* integration site represents the 10p15 site or the 10q11 site, especially since HA2(10p)11 retains both regions as shown in the microsatellite analysis. Microcell fusion using HA2(10p)11 and PC-3H generated sixteen PC deletion hybrids (PC11(10p) series). Three hybrids, PC11(10p)D, PC11(10p)E and PC11(10p)P, died *in situ* after isolation.

Molecular analysis of a majority of PC deletion hybrids was performed using microsatellite PCR Using informative markers, three hybrids (PC11(10p)A, PC11(10p)K, and PC11(10p)M) were identified that apparently contained the same introduced regions: an estimated 5.5 Mb region within 10p15, and a proximal region of approximately 8 Mb between 10p12.1 and p12.31. A fourth hybrid, PC11(10p)B, showed partial loss of the 10p15 region commonly retained in PC deletion hybrids A, K and M, as well as loss of two adjacent markers (D10S204 and D10S601) within the 10p12.1-p12.31 common region. In contrast, PC11(10p)L had lost most of this proximal region, retaining only one marker at 10p12.1 (D10S601) and maintaining the same distal region as PC deletion hybrids A, K and M. PC11(10p)G retained the introduced region 10p12.1-p12.31 (losing only marker D10S582) and demonstrated an absence of the distal region (retaining only the marker D10S1153). Finally, PC11(10p)N exhibited an almost complete lack of introduced 10p material, retaining primarily a region between 10p14-10p15, one marker at 10p15.3 (D10S559) and two markers at 10p12.1 (D10S111 and D10S204). However, additional markers proximal to D10S601 have not been tested. It is possible that all PC deletion hybrids contain some part of the proximal region of chromosome 10p, as well as some or all of the region from the centromere to 10q11. Until

markers that map to these regions are screened, it must be assumed, then, that these regions are retained in the PC deletion hybrids. Thus, the microcell transfer of a 10p fragment exhibiting a large interstitial deletion into PC-3H resulted in the generation of a panel of PC deletion hybrids with defined, overlapping deletions that were demonstrated by FISH and microsatellite analyses. These PC deletion hybrids were very informative when assayed for tumorgenicity *in vivo*.

The *in vivo* analysis of the PC deletion hybrids identified significant differences in tumorigenic potential. PC-3H, PC(22)2 (which contains an introduced chromosome 22 in the PC-3H background), PC(10p)D (10pter-q11 in the PC-3H background; Sanchez *et al*, 1996), and PC deletion hybrid cells were each subcutaneously injected (initially at 5 X 10^6 cells per animal, then at 1 X 10^7 cells per animal for subsequent assays) into five male athymic nude mice (4- to 6-weeks old). Tumor volumes were measured weekly and the tumors were excised eight weeks post-injection and established in culture. Three separate experiments are summarized in Table III. The parental PC-3H line was highly tumorigenic in all experiments, generating tumors in thirteen of the total fifteen animals injected, with an average tumor wet weight of 0.334 grams for 5 X 10^6 cells injected per animal, and average tumor wet weights in the range of 0.936-2.268 grams for at 1 X 10^7 cells injected per animal at fifty-six days post-injection. As shown previously (Sanchez *et al*, 1996), PC(10p)D demonstrated dramatic tumor suppression, and this was again illustrated in all three experiments (Table III). Tumorgenicity was strongly suppressed by PC deletion hybrids A, G and L. In all experiments suppression by these hybrids was equivalent to or better than that shown by PC(10p)D, as evidenced by the comparison of tumor wet weight data in Fig. 5. In contrast, PC deletion hybrids K, M and N were highly tumorigenic. PC deletion hybrids K and M, in particular, generated tumors of average volume and wet weight that surpassed tumors generated by the parental line PC-3H, for a difference of 3-5 times. PC deletion hybrid N was slightly less tumorigenic than K and M, but still generated an average tumor wet weight equivalent to or twice that of PC-3H. Interestingly, PC deletion hybrid B generated tumors that were intermediate in size and weight when compared to PC-3H and suppressed hybrids.

A comparison of the previously determined 10p regions transferred into the functionally suppressed and unsuppressed PC deletion hybrids assisted with the identification of the likely locations of tumor suppressor loci on chromosome 10p. Evaluation of hybrids A and L (both of which were suppressed for tumorigenesis *in vivo*) along with hybrid N (tumorigenic) suggested the location of tumor suppressor loci at two distinct regions, one within 10p15.3 and the other within the region 10p12.31 – q11 (see Figure 22). However, the significant tumorigenesis of hybrids K and M, which appear to contain the same introduced 10p regions as suppressed hybrid A, confounded the definitive identification of tumor suppressor loci within 10p15.3 or 10p12.1-q11. Molecular analysis using microsatellite PCR of explanted tumors generated by hybrids A, K and M showed no genetic difference to determine a genotype:phenotype correlation between these hybrids; the tumors generated by these hybrids apparently retained the introduced 10p regions initially determined before injection. The possibility of discrete deletions within 10p15.3 or 10p12.1-q11, or point mutations within the gene of interest at either region, therefore, remains open. Gene silencing mechanisms may also be responsible for these observations. Since markers to analyze the 10p11 and 10q11 regions were not used in the genetic analysis of the PC deletion hybrids, it may be possible to differentiate PC deletion hybrids K and M at the genetic level within the proximal region of 10p, and thus establish a genotype:phenotype correlation to explain the differences in tumor-suppressive phenotype between PC deletion hybrids A, K and M.

Since there was not a PC deletion hybrid retaining solely the 10p15.3 region, this region could not be tested independently for its ability to suppress tumor formation *in vivo*. Analysis of PC

deletion hybrid G, however, lent further credence for the location of a tumor suppressor locus at 10p12.31–q11. Hybrid G (which exhibited *in vivo* suppression) showed an essential lack of the introduced region 10p14-10p15.3 while retaining most of the proximal region beginning at 10p12.31. When compared to PC deletion hybrids L and N, hybrid G suggested the likely location for a tumor suppressor locus at 10p12.31-q11.

In addition, the analysis of PC11(10p)B suggested that the distal boundary of the candidate tumor suppressor locus within proximal 10p was between D10S204 and D10S601. The demonstration of partial tumorgenicity by hybrid B in conjunction with the loss of these markers - when compared to the data from hybrids A, L, N, and G – seemed to indicate a direct relationship with D10S601 retention and tumor suppression.

It must be emphasized that it is unknown how far proximally this 10p12 candidate region extends. Previous microsatellite analysis of the donor (HA2(10p)11) for these PC deletion hybrids showed retention from D10S211-D10S675. Until the PC deletion hybrids are screened with additional markers, it must be assumed that this candidate tumor suppressor region extends as far as 10q11, since the fragment in HA2(10p)11 originated from the subchromosomal region 10pter-q11.. The estimated size of the region between D10S211 and 10q11, based on the Human Genome Project Working Draft released 7 October 2000 (at genome.cse.ucsc.edu), is 31 Mb.

Analysis of the region bounded by D10S211-10q11 using GeneMap99 (www.ncbi.nlm.nih.gov/genemap) and the Chromosome 10 Sequencing Project Ensembl Annotation Server (www.ensembl.org/perl/mapview?chr=10) identified a subset of six known genes and at least thirty-two assembled expressed sequence tags (ESTs) that could represent novel genes. The candidate genes are shown in Table 4.

These results, then, regionally define a novel tumor suppressor locus involved in prostate cancer to 10p12.31-q11. Furthermore, with the availability of genome sequence information, we have identifed at least 6 genes and 32 EST clusters that map into this candidate locus.

Task 3-5. Task 3 was outlined to construct a BAC contig across the smallest region of nonoverlap between suppressed and unsuppressed hybrid clones. This task was accomplished by the Human Genome Project and information from genome databases was used to identify genes/ESTs in the candidate region. **Tasks 4 and 5** were to examine the expression of candidate genes in hybrid clones generated. Given the size of the region of interest, efforts to determine, gene-by-gene which genes are expressed in suppressed hybrids and not expressed in unsuppressed hybrids, are not feasible. Rather, we are constructing microarrays to examine gene expression differences in all genes/ESTs in this region and very soon, should have candidates for PAC-1 from our functionally defined region.

KEY RESEARCH ACCOMPLISHMENTS

* **Functional localization of a novel tumor suppressor locus for prostate cancer to within 31 Mb at chromosome 10p11-q11.**

* **Identification of all genes and ESTs in the genomic interval containing the tumor suppressor gene PAC-1**

* **Development of a Technology for Serial Microcell Fusion**

* **Development of a Novel Panel of Microcell Hybrid Clones for Functional Mapping at Chromosome 10p11.2-q11**

REPORTABLE OUTCOMES

Reportable outcomes of this research include the discovery hemizygosity for 10p markers in three different prostate cell lines, the development of novel reagents for mapping tumor suppressor loci within chromosome 10p in prostate cancer, the development of a panel of interstitutal deletion microcell hybrid clones containing multiple dominantly tagged selectable marker insertions of chromosome 10p for gene mapping and gene transfer, the limiting of a region of 31 Mb containing PAC-1, a novel tumor suppressor gene for prostate cancer, and the identification of all genes and ESTs in this region for further characterization as candidates for the tumor suppressor gene PAC-1.

In addition, Patricia Wong, a graduate student working on this project received stipend support from an NIH training grant in Molecular Genetics at M. D. Anderson. She also has won several awards for this research including the Sowell Huggins award, American Legion Auxiliary award, and AFLAC scholar award from the American Association of Cancer Research to present this research in platform session. Ms. Wong received a first place award for predoctoral research at the Texas Genetics society last year. Patty Wong received her doctorate in May, 2001. Her results are now being summarized for two manuscripts. Dr. Ann Killary is a member of the Prostate Cancer Multidisciplinary Program at M. D. Anderson headed by Dr. Andrew von Eshenbach, Dr. Josh Fidler, and Dr. Christopher Logothetis. She was also given a new laboratory last year on the seventh floor of a new research building, a floor dedicated to prostate cancer research at M. D. Anderson.

CONCLUSIONS

The inactivation of tumor suppressor genes is one of the features of the multistep progression characteristic of human malignancies. Chromosomal loss and subchromosomal deletion are among the several mechanisms by which this inactivation can occur. We have shown a reduction to hemizygosity within the chromosomal region 10p12-p15 in a prostatic cancer cell line derived from a bony metastasis, and furthermore demonstrate reversion to a tumor suppressive phenotype upon the introduction of the region 10p12.31-q11. We have also identified several candidate genes that map to this region.

In addition, we have identified four single-base changes located in an *Alu* element at 10p15.3. These changes were specific to PC-3H and gave a higher identity with the *Alu* element than the corresponding sequence found commonly in three male controls and two cell lines. The significance of these single-base changes within the context of the tumorigenicity of PC-3H is unknown, although an interesting comparison between this sequence and two functional estrogen-response elements was found. DNA sequencing of additional genomic DNA samples should give an indication of whether these changes are the result of rare single nucleotide polymorphisms. The alternative possibility that these changes could represent genomic alterations that could affect gene expression, especially in response to activated steroid hormone receptors, has yet to be determined.

We have also developed the technique of serial microcell fusion. This was a rapid way to generate small, defined fragments of the region 10pter-q11. Using this method, we have identified a functional tumor suppressor locus, PAC-1, within an estimated 31 Mb region from 10p12.31-q11. While the boundary within 10p12.31 is certain, the proximal boundary of the functionally

responsible region needs to be resolved by the microsatellite assay of the PC deletion hybrids used in this project.

Although the PC deletion hybrids that we generated in this study implicated the region 10p12.31-q11 as a functional tumor suppressor locus, these hybrids did not show definitive evidence for an additional locus within 10p15.3. Based on the data from one PC deletion hybrid, we propose a model in which a mild tumor suppressive effect is manifested by a postulated tumor suppressor locus at 10p15.3, while a demonstrative second locus within the proximal region of 10p (or the proximal region of 10q) exerts a stronger effect. However, we could not test the suppressive ability of the 10p15.3 region alone with the existing PC deletion hybrids. A recent review of the A9 deletion hybrids generated by our method of serial microcell fusion identified one hybrid, HA2(10p)8, that could potentially separate the two regions, 10p15.3 and 10p12.31-q11. HA2(10p)8 retains one marker within 10p15, with an apparent interstitial deletion of the remaining proximal markers used for analysis. Therefore, the proximal boundary extends beyond 10p12.31, and HA2(10p)8 may have lost PAC-1. Further microsatellite analysis to characterize the proximal and distal regions of the chromosome 10 fragment in this hybrid should determine its utility as a donor for microcell fusion with PC-3H, in order to test the ability of the 10p15.3 region to suppress tumorigenesis.

We have used a positional candidate approach to identify genes whose function may explain the tumor suppression observed in our PC deletion hybrids. Most interesting is the identification of CUL2, which maps to 10p11.22. Orthologs of the cullin family of proteins seem to participate in the regulation of the cell cycle through the degradation of key cell cycle proteins. No association between CUL2 and prostate cancer has yet been reported. Expression analysis of CUL2, as well as the other genes and ESTs that map to PAC-1, should significantly narrow the list of genes for further functional genomic analysis of tumor suppression in PC-3H.

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LIST OF PERSONNEL RECEIVING PAY FROM THE RESEARCH EFFORT

Ann Killary, Ph.D.

Mercedes Lovell

Patty Wong

TABLE 1. Summary of chromosomal gains and losses in PC-3H

CGH	
Gains (fraction of metaphases)	**Losses (fraction of metaphases)**
1p36.1-pter (3/3)	1p21-p31 (2/3)
1q21-q24 (3/3)	1q31(2/3)
1q31-qter (3/3)	2p12 (2/2)
3p14-p24 (3/4)	2q22-q24 (2/2)
3cen (3/4)	2q32-q33 (2/2)
3q13.3-q21 (3/4)	4p16-4cen (4/7)
3q26.1-qter (3/4)	4q21-qter (4/7)
5p15.3 (3/5)	5p13-p14 (3/5)
5q35 (3/5)	5q12-q31 (3/5)
6p23-p25 (3/5)	6q (3/5)
6p21.2-p22 (3/5)	7q21 (3/5)
7p22 (3/5)	8pter-q13 (4/5)
7q35-qter (3/5)	9 (3/5)
8q21.1-qter (4/5)	10p12-p15 (4/7)
10p12-q23 (4/7)	10q23-q26 (4/7)
11p15 (5/6)	12p11.2-p13 (4/6)
11cen-q14 (4/6)	12q21 (4/6)
11q14-qter (4/6)	13q14-qter (4/7)
12q22-qter (4/6)	14p11.2-pter (4/6)
13p11.2-q12 (4/8)	15p12-p13 (4/7)
14q11.2-qter (4/7)	15q15-q26 (4/7)
16p13.3-pter (2/3)	16p13.1-q11.2 (2/3)
17q21-qter (5/6)	17p12-q12 (4/6)
18pter-p11.1 (4/6)	18q11.2-q21 (4/7)
18q23 (4/6)	20q11.2 (4/6)
19 (3/5)	21p11.1-p12 (3/5)
20q13.1-qter (3/5)	22pter-q12 (4/6)
21q21-qter (3/5)	
22q13 (5/7)	

TABLE II. Summary of Microsatellite Analysis of PC-3H

Locus	# Alleles Present in PC-3H	Heterozygosity	Physical Distance from p terminus	Corresponds to Physical Map Location
D10S249	1	0.7513	0.15 Mb	10p15.3
D10S558	1	0.8100	0.67	10p15.3
D10S1145*	0	0.8750	0.67	10p15.3
D10S594	1	0.6948	1.60	10p15.3
D10S1716	1	0.7038	1.91	10p15.3
D10S602	1	0.7300	2.41	10p15.3
D10S1161	1	0.8330		
D10S1142	1	0.8330		
D10S514	1	0.8000		
D10S1706	1	0.7440		
D10S526	1	0.9091		
D10S1154	1	0.8330	2.87	10p15.3
D10S591	1	0.7124		
D10S1153	1	0.8120		
D10S179	1	0.8160		
D10S1152	1	0.8750		
D10S189	1	0.7318	6.8	10p14
D10S1779	1	0.8200	8.4	10p14
D10S1712	1	0.7019	9.0	10p14
D10S1720	1	0.6900	9.28	10p14
D10S585	1	0.6900		
D10S1705	1	0.7245	12.75	10p13
D10S570	1	0.8148	13.25	10p13
D10S191	1	0.8196	14.97	10p13
D10S674	1		17	10p13
D10S1477	1	0.7800		
D10S504	1	0.8333	18.21	10p12.33
D10S518	1	0.8333	20.37	10p12.33
D10S211	1	0.8438	22.92	10p12.31
D10S582	1	0.7627	25.53	10p12.1
D10S89	1	0.7450	27.32	10p12.1
D10S593	1	0.7300	28.4	10p12.1
D10S611	1		29	10p12.1
D10S600	1	0.8400	30	10p12.1
D10S204	1	0.7555	30.9	10p12.1
D10S601	1	0.8237	31.08	10p12.1

*** The initial analysis of D10S1145 showed a null allele and subsequent characterization of this locus is described in the text.**

TABLE III. Tumor incidence in PC hybrids 56 days postinjection

Experiment #	Cell line	Average tumor volume, mm^3 (average ± SD)	Average tumor wet weight, grams (average ± SD)	Number of cells injected	No. of tumors/ no. of mice injected
1	PC-3H	579.30 ±629.19	0.334 ± 0.398	5×10^6	3/5
1	PC(10p)D	233.00 ± 171.06	0.106 ± 0.077	5×10^6	4/5
1	PC11(10p)A	NPT	ND	5×10^6	0/5
1	PC11(10p)L	182.56 ± 180.49	0.092 ± 0.104	5×10^6	3/5
1	PC11(10p)N	981.29 ± 722.92	0.716 ± 0.522	5×10^6	4/5
1	PC11(10p)K	2008.79 ± 700.14	1.554 ± 0.725	5×10^6	5/5
1	PC11(10p)M	1258.73 ± 353.62	1.228 ± 0.407	5×10^6	5/5
1	PC(22)2	830.73 ± 1143.36	0.588 ± 0.821	5×10^6	2/5
2	PC-3H	2185.86 ± 549.43	2.268 ± 0736	1×10^7	5/5
2	PC(10p)D	774.14± 636.07	0.616 ± 0.400	1×10^7	5/5
2	PC11(10p)G	353.31 ± 403.70	0.502 ± 0.677	1×10^7 *	3/4
2	PC11(10p)N	3083.26 ± 1990.53	2.867 ± 1.213	1×10^7	5/5
2	PC11(10p)K	5876.52 ± 1238.87	6.695 ± 1.068	1×10^7	5/5
2	PC11(10p)M	5916.53 ± 1333.24	6.156 ± 1.588	1×10^7	5/5
2	PC(22)2	3053.66 ± 2328.14	2.878 ± 2.083	1×10^7	4/5
3	PC-3H	1416.75 ± 231.68	0.936 ± 0.180	1×10^7	5/5
3	PC(10p)D	412.95 ± 360.84	0.222 ± 0.225	1×10^7	4/5
3	PC11(10p)G	543.95 ± 248.35	0.310 ± 0.235	1×10^7	5/5
3	PC11(10p)L	312.15 ± 159.49	0.140 ± 0.086	1×10^7	5/5
3	PC11(10p)B	786.31 ± 360.70	0.506 ± 0.227	1×10^7	5/5
3	PC(22)2	2287.54 ± 608.12	1.664 ± 0.538	1×10^7	5/5

SD = standard deviation

NPT = no palpable tumors

ND = not determined

* Three mice were injected with 1×10^7 cells; 2/3 mice generated tumors. A fourth mouse was injected with a remaining 7.5×10^6 cells, which resulted in a tumor.

TABLE IV. A subset of candidate genes that map within PAC-1

Gene symbol	Gene name	LocusLink ID#
SVIL	supervillin	6840
MAP3K8	mitogen-activated protein kinase kinase kinase 8	1326
KIF5B	kinesin family member 5B	3799
ITGB1	integrin, beta 1 (fibronectin receptor, beta polypeptide, antigen CD29 includes MDF2, MSK12)	3688
CREM	cAMP responsive element modulator	1390
CUL2	cullin 2	8453

LocusLink is available at www.ncbi.nlm.nih.gov.

Figure 1. **Primer map used to limit the putative homozygous deletion in PC-3H.** A bacterial artificial chromosome (BAC) containing the human genomic region including D10S1145 was identified (RPCI-11 164C1), and its DNA sequence used to design PCR primers to limit the putative homozygous deletion of D10S1145 in PC-3H. **A.** D10S1145 primers (black) generate a 250 to 400-bp PCR product, which is absent in PC-3H. **B.** Primers (blue) designed to amplify a 160-bp PCR product from a region 214 bases downstream of D10S1145 (black) and an adjacent *Alu* element (in red, oriented as shown). **C.** Primers (violet) designed to amplify a 147-bp PCR product from a region overlapping and upstream of D10S1145 (black). **D.** Primers (green) designed to amplify a 465 to 615-bp product from the region spanning the *Alu* element and the polymorphic repeat of D10S1145.

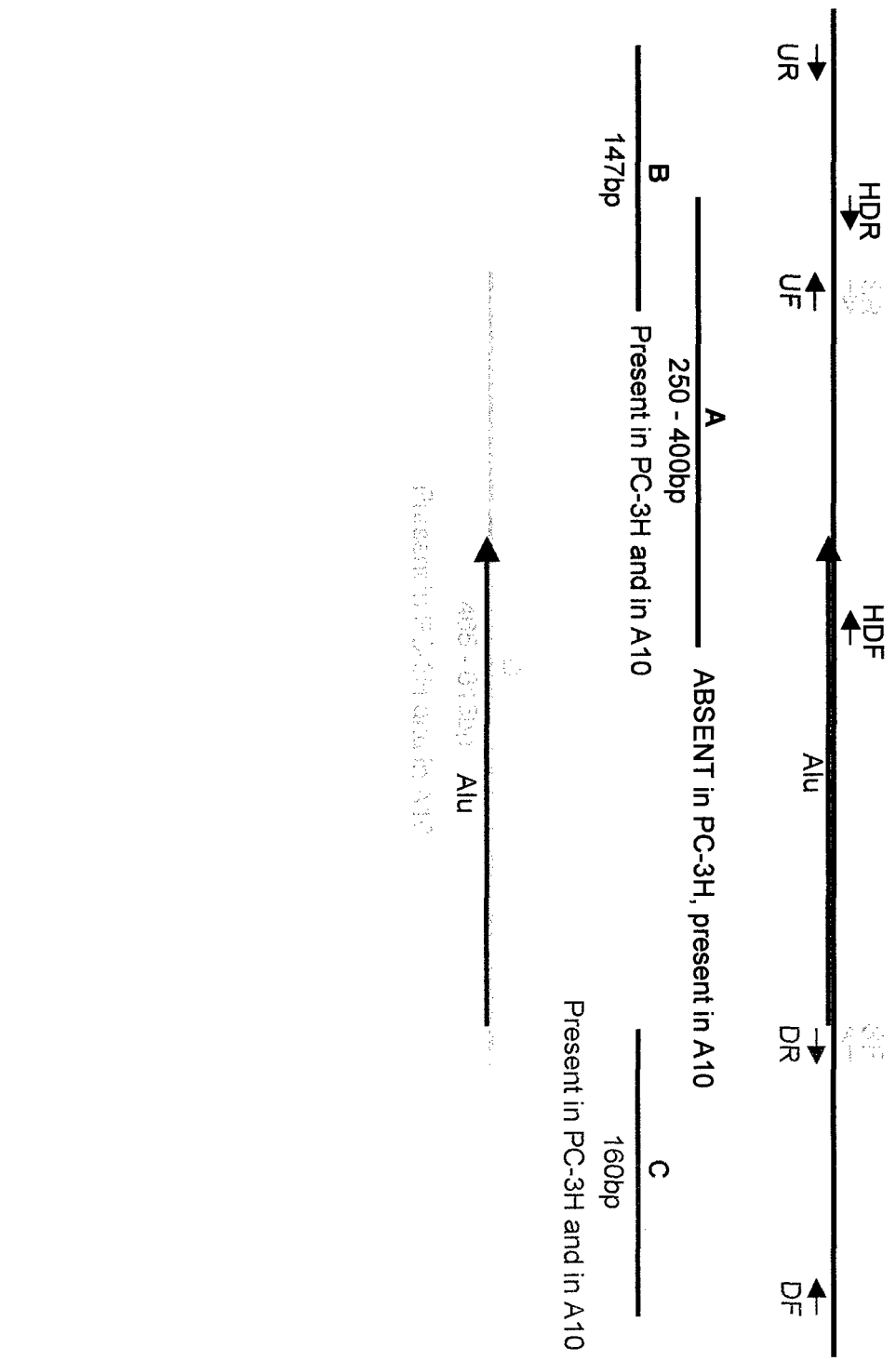
UR
HDR
UF
A
250 - 400bp
ABSENT in PC-3H, present in A10
B
147bp
Present in PC-3H and in A10
HDF
Alu
DR
C
160bp
Present in PC-3H and in A10
DF
Alu

Figure 1

Figure 2. **DNA sequence of a 159-bp region within an *Alu* element shows four single-base changes in PC-3H when compared to three male DNA controls and two cell lines.** Sequence analysis of the region adjacent and downstream of D10S1145 identified four single-base changes in PC-3H (in pink and boxed) when compared to the corresponding DNA sequence commonly found in three normal male controls and two cell lines. This 159-bp region is part of the 274-bp region that is 90% identical to the *Alu*-Sx subfamily, and resides just downstream of D10S1145.

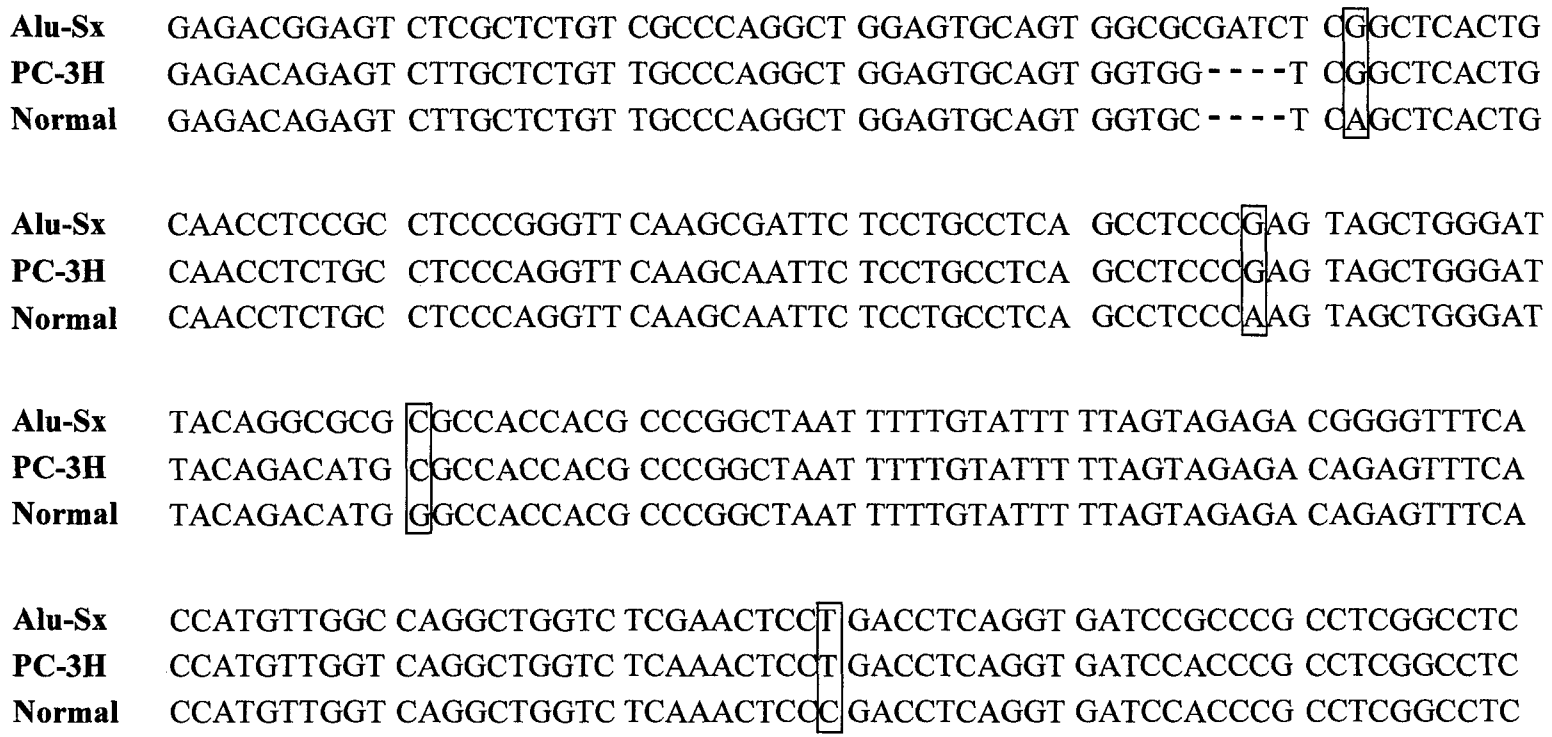
Alu-Sx GAGACGGAGT CTCGCTCTGT CGCCCAGGCT GGAGTGCAGT GGCGCGATCT CGGCTCACTG
PC-3H GAGACAGAGT CTTGCTCTGT TGCCCAGGCT GGAGTGCAGT GGTGG----T CGGCTCACTG
Normal GAGACAGAGT CTTGCTCTGT TGCCCAGGCT GGAGTGCAGT GGTGC----T CAGCTCACTG
Alu-Sx CAACCTCCGC CTCCCGGGTT CAAGCGATTC TCCTGCCTCA GCCTCCCGAG TAGCTGGGAT
PC-3H CAACCTCTGC CTCCCAGGTT CAAGCAATTC TCCTGCCTCA GCCTCCCGAG TAGCTGGGAT
Normal CAACCTCTGC CTCCCAGGTT CAAGCAATTC TCCTGCCTCA GCCTCCCAAG TAGCTGGGAT
Alu-Sx TACAGGCGCG CGCCACCACG CCCGGCTAAT TTTTGTATTT TTAGTAGAGA CGGGGTTTCA
PC-3H TACAGACATG CGCCACCACG CCCGGCTAAT TTTTGTATTT TTAGTAGAGA CAGAGTTTCA
Normal TACAGACATG GGCCACCACG CCCGGCTAAT TTTTGTATTT TTAGTAGAGA CAGAGTTTCA
Alu-Sx CCATGTTGGC CAGGCTGGTC TCGAACTCCT GACCTCAGGT GATCCGCCCG CCTCGGCCTC
PC-3H CCATGTTGGT CAGGCTGGTC TCAAACTCCT GACCTCAGGT GATCCACCCG CCTCGGCCTC
Normal CCATGTTGGT CAGGCTGGTC TCAAACTCCC GACCTCAGGT GATCCACCCG CCTCGGCCTC

Figure 2

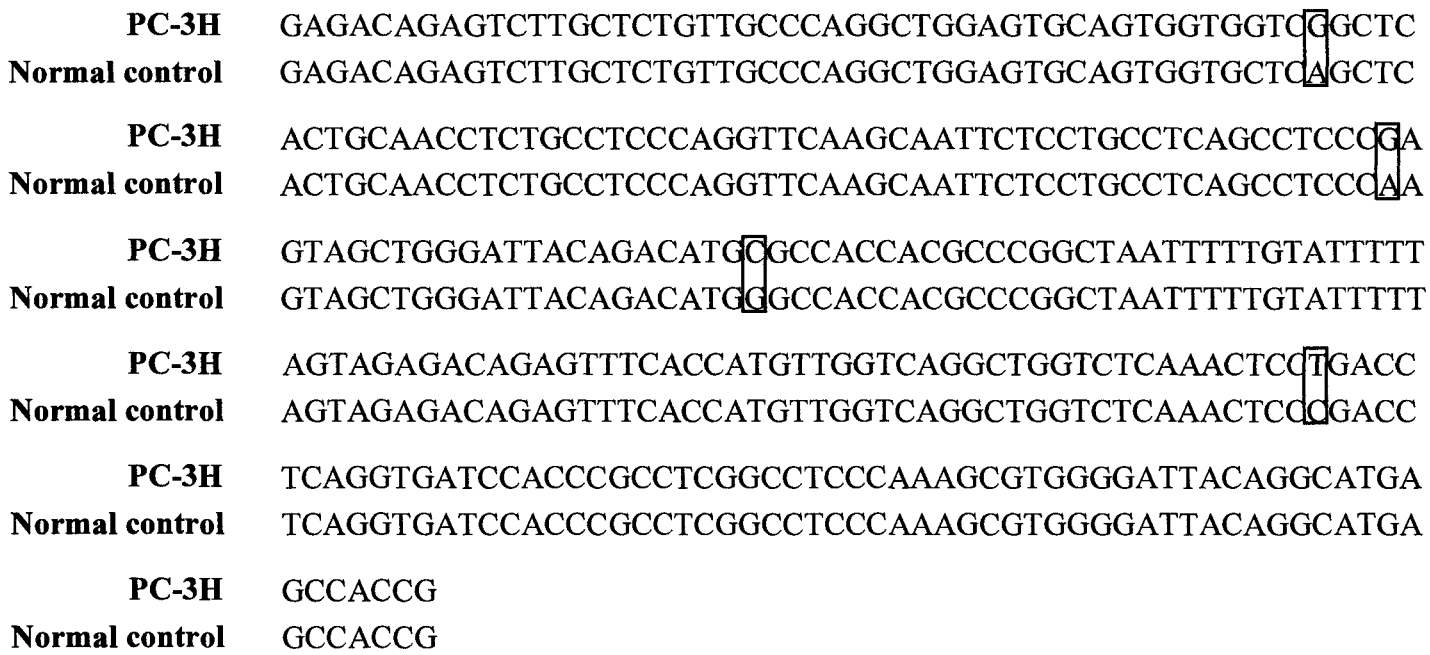
PC-3H GAGACAGAGTCTTGCTCTGTTGCCCAGGCTGGAGTGCAGTGGTGGTCGGCTC
Normal control GAGACAGAGTCTTGCTCTGTTGCCCAGGCTGGAGTGCAGTGGTGCTCAGCTC
PC-3H ACTGCAACCTCTGCCTCCCAGGTTCAAGCAATTCTCCTGCCTCAGCCTCCCGA
Normal control ACTGCAACCTCTGCCTCCCAGGTTCAAGCAATTCTCCTGCCTCAGCCTCCCAA
PC-3H GTAGCTGGGATTACAGACATGCGCCACCACGCCCGGCTAATTTTTGTATTTTT
Normal control GTAGCTGGGATTACAGACATGGGCCACCACGCCCGGCTAATTTTTGTATTTTT
PC-3H AGTAGAGACAGAGTTTCACCATGTTGGTCAGGCTGGTCTCAAACTCCTGACC
Normal control AGTAGAGACAGAGTTTCACCATGTTGGTCAGGCTGGTCTCAAACTCCCGACC
PC-3H TCAGGTGATCCACCCGCCTCGGCCTCCCAAAGCGTGGGGATTACAGGCATGA
Normal control TCAGGTGATCCACCCGCCTCGGCCTCCCAAAGCGTGGGGATTACAGGCATGA
PC-3H GCCACCG
Normal control GCCACCG

Figure 2

PC-3H	TTTCACCATG TTGGTCAGGC TGGTCTCAAA CTCCTGACCT CAGGTGATCC
Normal	TTTCACCATG TTGGTCAGGC TGGTCTCAAA CTCCCGACCT CAGGTGATCC
ERF-3	TTTCACCATG TTGGTCAGGC TGGTCTCAAA CTCCTGACCT C - - GTGATCC
BCER-1	TTTCACCATA TTGGTCAGGC TGGTCTCGAA CTCCTGACCT CAGGTGATCC
***Alu*-Sx**	TTTCACCATG TTGGCCAGGC TGGTCTCGAA CTCCTGACCT CAGGTGATCC
***Alu*-Sp**	TTTCTCCATG TTGGTCAGGC TGGTCTCGAA CTCCCGACCT CAGGTGATCC
HS *Alu*	TTTCACCGTT TTAGCCGGGA TGGTCTCGAT CTCCTGACCT C - - GTGATCC

Figure 3. **Serial microcell fusion.** In any microcell fusion experiment, it is possible to generate hybrids containing fragments of the introduced chromosomal material. For this reason, we devised the serial transfer of human 10pter-q11 from one A9 background into another to generate defined deletions of this subchromosomal region. Colcemid-induced micronucleation of HA(10p)A and the subsequent extrusion of microcells in the presence of cytochalasin B allowed the introduction of the *neo*-tagged (green) chromosomal fragment into fresh A9 cells using microcell fusion. Resulting HA2(10p) series clones were grown in G418 to selectively retain the *neo*-tagged fragment. HA2(10p) clones were than analyzed using microsatellites and FISH to determine the presence of 10p fragments in the A9 background.

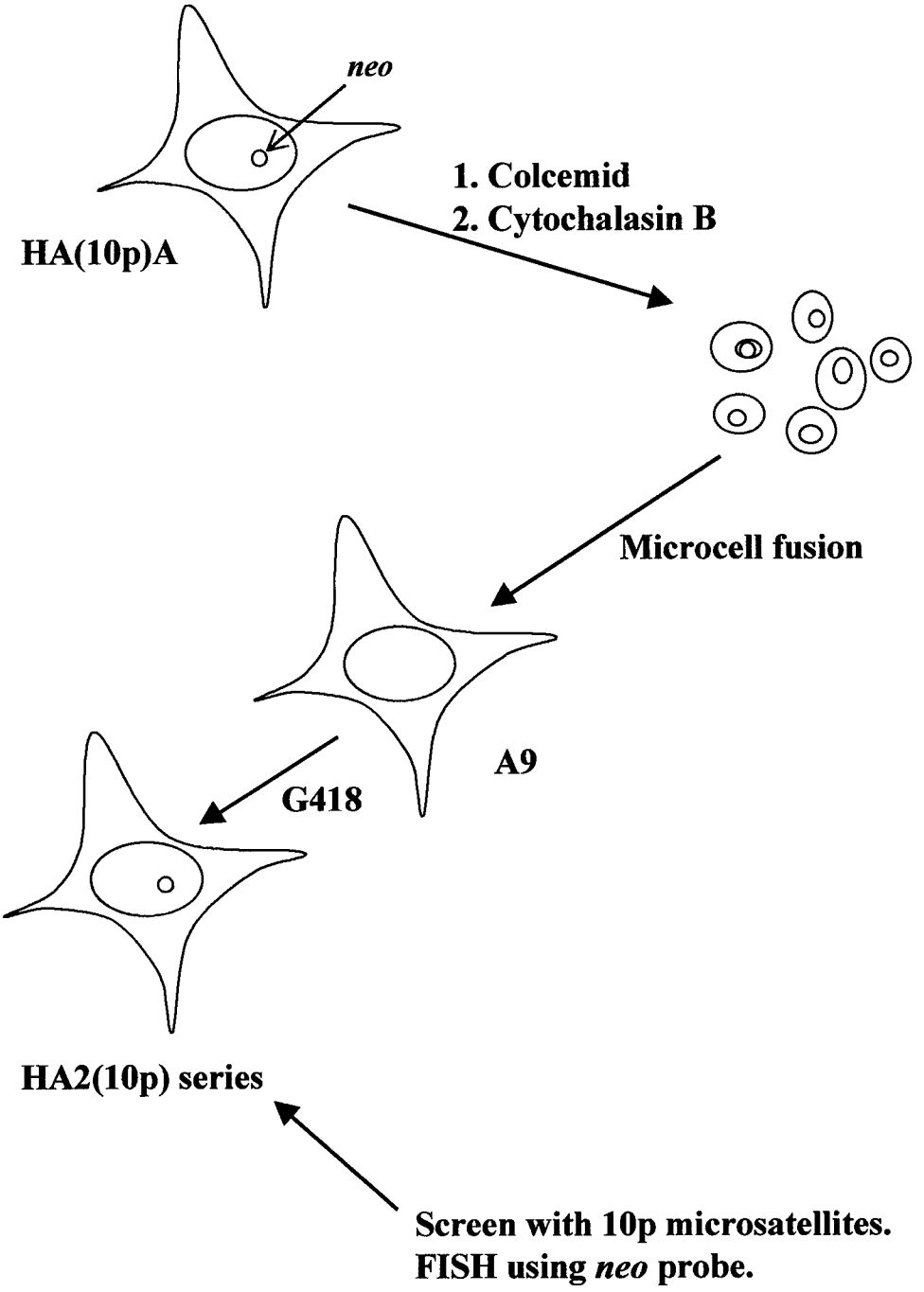
neo
HA(10p)A
1. Colcemid
2. Cytochalasin B
Microcell fusion
A9
G418
HA2(10p) series
Screen with 10p microsatellites.
FISH using neo probe.

Figure 3

Figure 4. **Panel of five HA2(10p) hybrids with defined, overlapping interstitial deletions of 10p.** ● = presence of the locus; ○ = absence of the locus. HA2(10p)11 demonstrates a large interstitial deletion of approximately 20 Mb. HA2(10p)27 has a smaller deletion of an estimated 14 Mb. HA2(10p)2, 19 and 33 were excluded from further analysis.

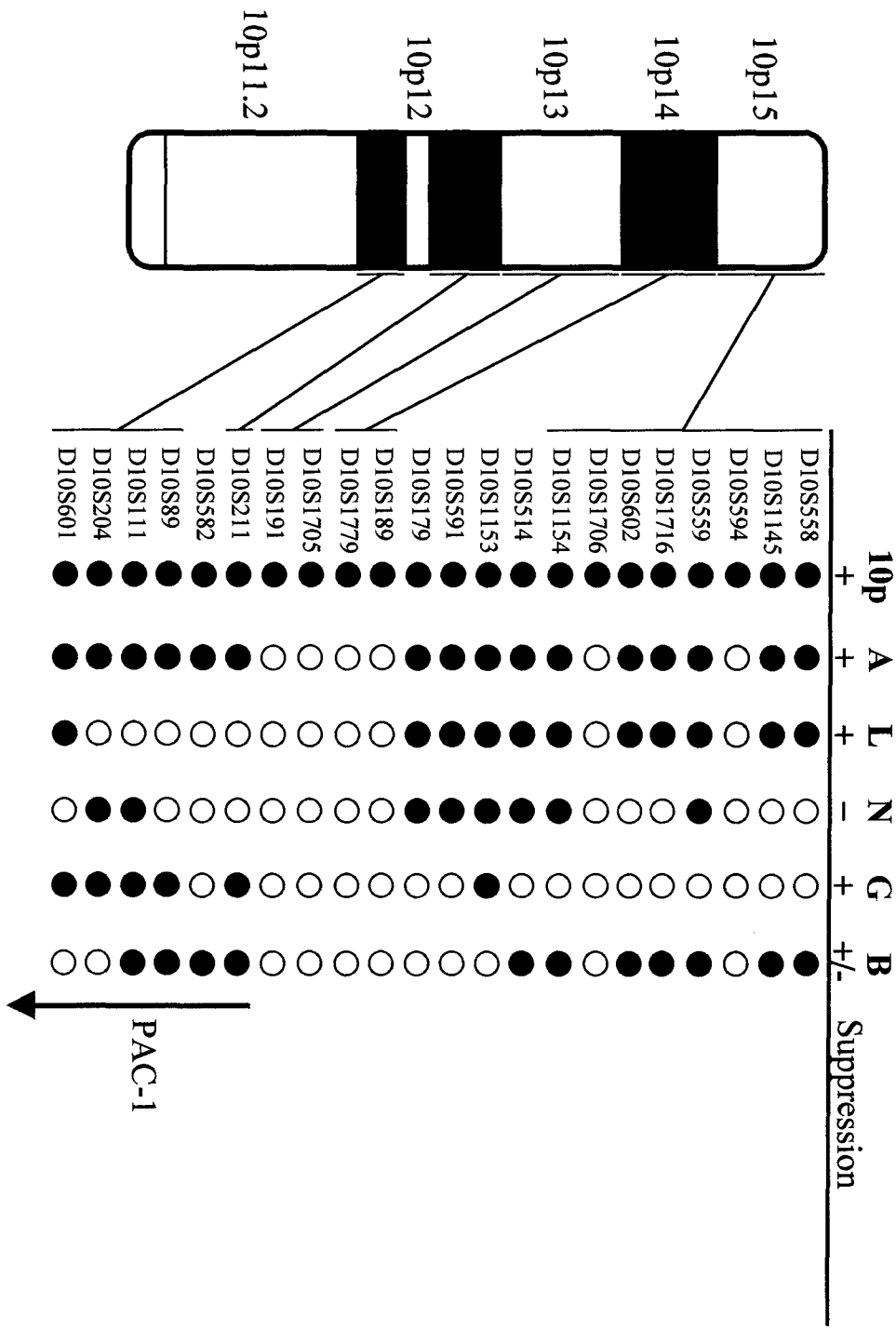
10p15
10p14
10p13
10p12
10p11.2
10p
A
L
N
G
B
+/-
Suppression
D10S558
D10S1145
D10S594
D10S559
D10S1716
D10S602
D10S1706
D10S1154
D10S514
D10S1153
D10S591
D10S179
D10S189
D10S1779
D10S1705
D10S191
D10S211
D10S582
D10S89
D10S111
D10S204
D10S601
PAC-1

Figure 4

Figure 5. **Tumor wet weights determined 56 days postinjection of PC11(10p) series hybrids.** **A.** PC11(10p)L demonstrates suppression comparable to PC(10p)D, which contains an introduced 10pter-q11 fragment. In contrast, hybrids N, K and M generate tumors of average weights comparable to those generated by the parental PC-3H cell line, or two to three times greater. **B.** Hybrid G exhibits tumor suppression, while hybrids N, K and M reliably reproduce the previous experiment's results. **C.** Hybrid B demonstrates intermediate tumor suppression, generating tumors that were larger than those generated by suppressed hybrid PC(10p)D, but smaller than those produced by the parental PC-3H cell line.

C.

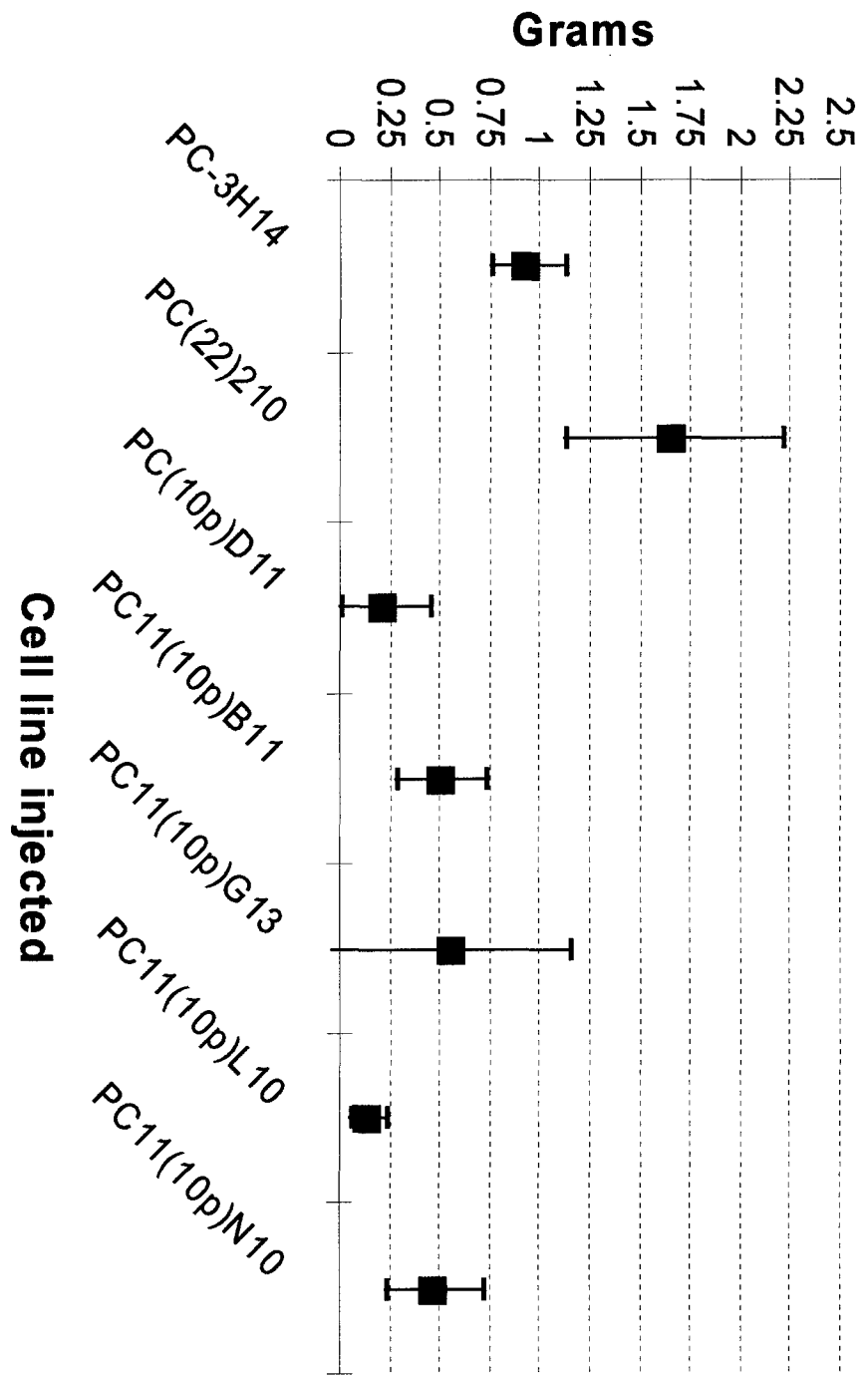
Grams
0
0.25
0.5
0.75
1
1.25
1.5
1.75
2
2.25
2.5
PC-3H14
PC(22)210
PC(10p)D11
PC11(10p)B11
PC11(10p)G13
PC11(10p)L10
PC11(10p)N10
Cell line injected

Figure 5

DEPARTMENT OF THE ARMY
US ARMY MEDICAL RESEARCH AND MATERIEL COMMAND
504 SCOTT STREET
FORT DETRICK, MARYLAND 21702-5012

REPLY TO
ATTENTION OF:

MCMR-RMI-S (70-1y) 8 Jan 2003

MEMORANDUM FOR Administrator, Defense Technical Information Center (DTIC-OCA), 8725 John J. Kingman Road, Fort Belvoir, VA 22060-6218

SUBJECT: Request Change in Distribution Statement

1. The U.S. Army Medical Research and Materiel Command has reexamined the need for the limitation assigned to the enclosed. Request the limited distribution statement for the enclosed be changed to "Approved for public release; distribution unlimited." These reports should be released to the National Technical Information Service.

2. Point of contact for this request is Ms. Judy Pawlus at DSN 343-7322 or by e-mail at judy.pawlus@det.amedd.army.mil.

FOR THE COMMANDER:

Encl

PHYLLIS M. RINEHART
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