The use of multicolor fluorescence technologies in the characterization of prostate carcinoma cell lines: a comparison of multiplex fluorescence in situ hybridization and spectral karyotyping data

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Abstract

Recent studies have identified several chromosome regions that are altered in primary prostate cancer and prostatic carcinoma cell lines. These targeted regions may harbor genes involved in tumor suppression. We used multiplex fluorescence in situ hybridization (M-FISH) to screen for genetic rearrangements in four prostate cancer cell lines, LNCaP, LNCaP.FCG, DU145, and PC3, and compared our results with those recently obtained using spectral karyotyping (SKY). A number of differences was noted between abnormalities characterized by SKY and M-FISH, suggesting variation in karyotype evolution and characterization by these two methodologies. M-FISH analysis showed that hormone-resistant cell lines (DU145 and PC3) contained many genetic alterations (>15 per cell), suggesting high levels of genetic instability in hormone-refractory prostate cancer. Most chromosome regions previously implicated in prostate cancer were altered in one or more of these cell lines. Several specific chromosome aberrations were also detected, including a del(4)(p14) and a del(6)(q21) in the hormone-insensitive cell lines, a t(1;15)(p?;q?) in LNCaP, LNCaP, and PC3, and a i(5p) in LNCaP.FCG, DU145, and PC3. These clonal chromosome abnormalities may pinpoint gene loci associated with prostate tumourigenesis, cancer progression, and hormone sensitivity. © 2001 Elsevier Science Inc. All rights reserved.

1. Introduction

Prostate cancer is the most commonly diagnosed, and the second leading cause of death from cancer in North American and European men [1]. Although most patients with prostate cancer have an initial favorable response to hormone therapy, most ultimately progress to hormone resistance, within 2 years for patients with metastatic disease and within 4 years for patients with localized disease [2]. A positive family history of prostate cancer is a risk factor for the development of this disease, and there are at least three genes that have been identified in these families [3]. The complexity of genetic events in prostate cancer development is demonstrated by the discovery of alterations to the p53 and retinoblastoma (Rb) genes, LOH at the DCC locus, and over expression of ras and myc in prostate cancer tissues [4–8]. Use of cytogenetic techniques has demonstrated loss of 6q, 9p, 13q, and 16q; trisomy 7, 8, 12, 17, and X; and LOH of 16 in carcinoma of the prostate [9–16]. The ultimate discovery of how these genes are involved in the regulation of tumor biology and hormone sensitivity would be a major step forward in the understanding and management of prostate cancer. Conventional cytogenetic analysis on primary tissue from patients with prostate cancer has been hampered by difficulties in obtaining adequate tissue with sufficient mitotic cells. Establishing cell lines has been equally difficult and as a consequence only four are widely available for study: LNCaP, LNCaP.FCG, DU145, and PC3 [17–20].

Recent advances in molecular technology have had considerable impact on cytogenetic analysis, allowing greater resolution and accuracy. Significant advances in fluorescence technology include the development of multiplex FISH (M-FISH) and spectral karyotyping (SKY). M-FISH is a combinatorial technique that allows the identification of human chromosomes by ‘painting’ them with a spectrum of DNA probes labeled with a unique combination of five fluorochromes [21]. Sophisticated image analysis applies the relevant pseudocolor to allow visualization in 24 discrete colors.
M-FISH was initially used to investigate hematological malignancies [22,23]. Spectral karyotyping is a technique that uses the same combinatorial approach to probe production, but uses an interferometer to convert the fluorescent image simultaneously into spectra for each individual pixel. These pixel spectra are captured on a CCD array and converted to multicolor images using Fourier transformation. Spectral karyotyping has been previously used to characterize hematologic malignancies, breast carcinoma, and bladder cancer [24–28], as well as prostate cancer [29,30]. Both these procedures allow each of the 24 human chromosomes to be identified simultaneously in 24 discrete colors, making it possible to screen for chromosome rearrangements between all chromosomes. There is increasing recognition of its potential in solid cancers, including prostate cancer, which have been largely limited to conventional cytogenetics and some molecular techniques. The study reported here is the first attempt to characterize the chromosomal rearrangements occurring in these cell lines using M-FISH to assess the degree of extra information that can be obtained over conventional cytogenetics and to assess its further potential to investigate the abnormalities associated with tumor progression and loss of hormone sensitivity. This report also compares these M-FISH results to the spectral karyotyping data previously published [29,30] to ascertain the advantages and limitations of these two technologies.

2. Methods

2.1. Cell culture and harvest

The prostate carcinoma cell lines, LNCaP (passage 30) and DU145 (passage 30), were supplied by the Imperial Cancer Research Fund (London, UK), and LNCaP.FCG (passage 25) and PC3 (passage 30) were supplied by European Collection of Cell Cultures (CAMR, Salisbury, UK). The cell lines DU145, LNCaP, and LNCaP.FCG were cultured in RPMI + 10% foetal calf serum (FCS) with 45 IU/ml penicillin and 45 µg/ml streptomycin. The cell line PC3 was cultured in Coon’s modified Ham’s F12 + 7% FCS with 2 mM glutamine, 45 IU/ml penicillin, and 45 µg/ml streptomycin. All cell lines were incubated at 37°C in culture flasks with an atmosphere of 5% carbon dioxide in air.

Metaphase slide preparations were made by adding colcemid (0.015 µg/ml; GIBCO, Parsley, UK), for 2 to 4 hours, treating with hypotonic solution (0.075M potassium chloride for 10 minutes at 37°C), then fixing cells with methanol:acetic acid (3:1) and spreading onto slides.

2.2. Cytogenetic analysis

M-FISH was carried out according to standard manufacturers instructions (SpectraVysion Probes, Vysis, London, UK). M-FISH was visualized by fluorescence microscopy using an Olympus Provis (Olympus, London, UK) with an M-FISH filter configuration (Vysis, London, UK). M-FISH images were captured with a Sensys Camera (Photometric Perceptive Scientific Inc., Chester, UK) and analyzed using M-FISH software (Perceptive Scientific Inc., Chester, UK). Ten M-FISH karyotypes were analyzed for each cell line. Rearrangements were classed as clonal when detected in two or more metaphase cells [31]. All chromosome rearrangements detected using M-FISH were confirmed by conventional painting with total chromosome DNA probes (Appligene Oncor, UK). G-banded analysis was achieved using standard methods [32,33] and used to confirm deletions and rearrangements.

3. Results

3.1. M-FISH, conventional painting, and G-banding results

M-FISH analysis of these cell lines elucidated a significant number of clonal rearrangements and characterized several markers initially observed with only conventional cytogenetics. A number of nonclonal chromosome aberrations were seen, but only the clonal abnormalities (seen in two or more metaphase cells) are described here. The M-FISH karyotypes for each cell line are shown in Figs. 1 through 4. Clonal abnormalities for each cell line are displayed in Table 1. Two-color chromosome painting was used to confirm rearrangements detected with M-FISH and G-banding was used to confirm all other abnormalities. Figs. 5 and 6 show examples of chromosome painting to confirm M-FISH detected abnormalities.

LNCaP (Fig. 1) and LNCaP.FCG (Fig. 2) shared several apparently identical chromosome rearrangements, reflecting their similar underlying origins. Both cell lines contained between two and four copies of each autosome and either one or two copies of the sex chromosomes. Rearrangements common to LNCaP and LNCaP.FCG included: t(1;15)(p?;q?),t(6;16)(p?;q?),der(6)t(4;6), and add(4)(wcp4, wcp6, wcp10+). M-FISH analysis also detected structural aberrations where no apparent exchange of material was observed. These aberrations were confirmed using conventional G-banded analysis. All LNCaP (10/10) metaphases analyzed contained del(2)(p16), although this abnormality was only detected in 80% (8/10) metaphases in LNCaP.FCG. From LNCaP and LNCaP.FCG, 30% and 40% of metaphases, respectively, displayed a del(10)(q24). Metaphase cells from both LNCaP and LNCaP.FCG showed either one or two copies of the rearrangements. LNCaP and LNCaP.FCG also displayed abnormalities not shared between cell lines that may reflect the underlying differences in their physiologies. In 30% of cells, LNCaP contained multiple copies of a del(12)(?) and a del(13)(q21.1) in 50% of metaphase cells analyzed. Abnormalities seen in LNCaP.FCG included a der(9)(t(5;9), a der(13)(t(13;19), and an i(5p)(p10), seen in 70%, 30%, and 40% of cells, respectively.

The DU145 (Fig. 3) cell line contained more clonal abnormalities than both LNCaP cell lines. The clonal abnormalities observed in DU145 are shown in Table 1. Most rearrangements occurred in all cells examined, although the
der(9)del(9), der(10), and the der(Y) were seen in 70%, 80%, and 60%, respectively. Aberrations confirmed by G-banding included del(4)(p14), del(6)(q21), del(9)(p22), and del(11)(q23). Using conventional G-banding, the der(15)(?) was identified as a derivative chromosome 15 resulting from two pairs of satellites.

Like DU145, PC3 (Fig. 4) exhibited a greater number of rearrangements than the LNCaP cell lines (Table 1). These rearrangements were detected in all cells examined, with the exception of the add(1)(wcp15+,wcp1+,wcp10+;30%) and add(15)(wcp8+,wcp15+,wcp1+,wcp10+;30%).

Figs. 5 and 6 show conventional painting on PC3 to confirm the origins of several large derivative chromosomes. Fig. 5 demonstrates the origin of several derivatives involving chromosome 8 and 12 material, whereas Fig. 6 demonstrates the origins of two markers derived from chromosomes 1 and 10.

3.2. Comparison of previously published SKY results and the present M-FISH data

Spectral karyotyping has recently been used to characterize three of the four prostate carcinoma cell lines used in the present study [29,30]. Table 1 shows examples of derivative chromosomes detected in each study. The M-FISH analysis of the LNCaP cell line described here closely matches the previously published data. All studies demonstrated a rela-
tively low level of rearrangement in the LNCaP cell line (less than 10 abnormalities per genome). All three studies detected and characterized the reciprocal translocations between chromosomes 1 and 15 and chromosomes 6 and 16. All three studies also detected deletions of chromosomes 2 (p13), 10(q23), and 13(q21.1). Pan et al. [29] detected a translocation between chromosome 4 and 6. The M-FISH data and previously published data [30] also characterized this translocation, but in addition detected material from chromosome 10 on the q arm of the derivative chromosome 4. M-FISH data shows the presence of two additional markers derived from chromosome 12 (Table 1) not detected previously.

Several consistent clonal changes were observed in all three studies of the prostate carcinoma cell line, DU145. Each study detected between 15 and 20 abnormalities per diploid cell. Abnormalities detected in all three studies included t(Y;20), t(1;4), t(5;21), t(11;12), t(2;13), t(14;18), and an i(5p). A derivative chromosome 13 was observed in all three studies and shown to contain material from chromosomes 2 and 13. M-FISH also revealed an insertion of chromosome 16 material in this derivative chromosome.
Both previously published studies detected a derivative chromosome 7, t(7;8), not detected using M-FISH analysis. Work presented here and data published by Beheshti et al. [30] described a der(14)t(3;14), whereas Pan et al. [29] and this present M-FISH study both described a der(16)t(6;16). Each study also detected specific clonal abnormalities only observed in one of the three studies. Clonal abnormalities specifically detected with M-FISH are highlighted in Table 1. Beheshti et al. [30] and Pan et al. [29] also classified clonal abnormalities specific to their studies.

All three studies detected relatively high levels of rearrangement in the cell line, PC3 (30+ abnormalities per diploid cell). Clonal chromosome abnormalities detected by all studies included a der(2)t(2;8), a der(4)t(4;6), a der(4)t(4;10), an i(5)(p10), and a der(12)t(8;12). All three studies also characterized a derivative chromosome 10 involving relatively large segments of chromosome 3 material. Pan et al. [29] characterized an add(10)(wcp3+,wcp10+,wcp3+; Fig. 7 C iv), whereas using the current M-FISH, and Beheshti et al. [30] described a more complex derivative chromosome. This M-FISH study demonstrated chromosome 1 material within the chromosome 10 segment of this marker (Fig. 7, C iv), which was confirmed on conventional chromosome painting (Fig. 6), whereas Beheshti et al. [30] showed the presence of chromosome 4 material in this derivative chromosome (Fig. 7, C iv). This M-FISH analysis and the SKY analysis of Beheshti et al. [30] both characterized a del(6)(q21–q26) as well as two similar markers involving the centromere of chromosome 1 (Fig. 7, C ii). These two markers showed identical morphologic features in both these studies, except that M-FISH identified chromosome 10 material in the marker, whereas SKY analysis [30] classified this material as chromosome 2 in origin. Conventional painting confirmed the M-FISH analysis. Pan et al. [29] and Beheshti et al. [30] demonstrated the presence of a add(2)(wcp2+,wcp15+,wcp17+), a der(3)t(3;15), a der(4) t(4;12), and a der(15)t(5;15). A deletion of chromosome 3 was observed using M-FISH of similar size to the der(3) observed by the other two SKY studies. Conventional painting of this marker demonstrated no chromosome 15 material. M-FISH and SKY analysis [29] both observed a der(14)t(14;15). The prostate cell line, PC3, exhibited a relatively large quantity of clonal abnormalities specific to each individual study discussed here (M-FISH data in Table 1). In PC3, Beheshti et al. [30] and Pan et al. [29] classified several derivative chromosomes specific to their SKY studies.

4. Discussion

LNCaP [17,34] and LNCaP.FCG [18,35] are hormone-sensitive cell lines, whereas DU145 [19] and PC3 [20] are hormone insensitive. LNCaP.FCG was derived from a fast-growing colony of the original LNCaP parental cells. Conventional G-banded analysis has been achieved on these cell lines, but several abnormal chromosomes could not be characterized [19,20,34,35]. Using conventional and molecular cytogenetics, the prostate carcinoma cell line LNCaP has been shown to contain a t(6;16)(p21;q22) [36] and abnormalities of chromosome 8 [37], 10 [38], and 5 [39]. In this study, M-FISH was used to characterize fully the four prostate carcinoma cell lines, LNCaP, LNCaP.FCG, DU145, and PC3. Several novel clonal rearrangements have been identified in each cell line, some of which occur in more than one cell line. LNCaP and LNCaP.FCG exhibited several similar clonal rearrangements previously described [29,30,38].
Table 1
Clonal structural abnormalities observed by M-FISH in each cell line

<table>
<thead>
<tr>
<th>LNCaP</th>
<th>LNCaP.FCG</th>
<th>DU145</th>
<th>PC3</th>
</tr>
</thead>
<tbody>
<tr>
<td>t(1;15)(p?;q?)</td>
<td>t(1;15)(p?;q?)</td>
<td>der(1)t(1;4)(wcp4+,wcp1+)</td>
<td>der(1)(wcp8+,wcp12+,wcp1+, wcp10+)</td>
</tr>
<tr>
<td>wcp15+,wcp15+,wcp1+</td>
<td>wcp15+,wcp1+</td>
<td></td>
<td>der(1)(wcp15+,wcp1+,wcp10+)</td>
</tr>
<tr>
<td>del(2)(p16)(wcp2+)</td>
<td>del(2)(p16)(wcp2+)</td>
<td>der(2)(t;2)(wcp2+,wcp4+)</td>
<td>der(2)(t;28)(wcp2+,wcp8+)</td>
</tr>
<tr>
<td>add(4)(wcp4+,wcp6+,wcp10+)</td>
<td>add(4)(wcp4+,wcp6+,wcp10+)</td>
<td>del(3)(wcp3+)</td>
<td>der(4)(t;4)(wcp6+,wcp4+)</td>
</tr>
<tr>
<td>del(9)(p22)(wcp9)</td>
<td>del(9)(p22)(wcp9)</td>
<td>der(4)(t;4)(wcp6+,wcp4+)</td>
<td>der(4)(t;21)(wcp21+,wcp4+)</td>
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<td>der(9)t(9;15)(wcp9)</td>
<td>der(9)t(9;15)(wcp9)</td>
<td>der(4)(del4)(p14)t(3;4)(wcp4+,wcp3+)</td>
<td>der(4)(t;4)(wcp10+,wcp4+)</td>
</tr>
<tr>
<td>der(12)t(12;12)+</td>
<td>del(10)(q24)(wcp10+)</td>
<td>der(9)(del9)(p12)t(9;15)(wcp9+,wcp15+)</td>
<td>der(10)(t;21)(wcp21+,wcp10+)</td>
</tr>
<tr>
<td>del(1)(wcp10+)</td>
<td>del(10)(q24)(wcp10+)</td>
<td>del(10)(t;21)(wcp21+,wcp10+)</td>
<td>i(10)(?)+add(10)(wcp3+,wcp10+,wcp1+,wcp10+)</td>
</tr>
<tr>
<td>del(13)(q21.1)(wcp13)+</td>
<td>del(13)(q21.1)(wcp13)+</td>
<td>der(12)t(8;12)(wcp12+,wcp8+)</td>
<td>add(10)(wcp14+,wcp10+,wcp5+)</td>
</tr>
<tr>
<td>add(13)(wcp13+,wcp16+,wcp2+)</td>
<td>add(13)(wcp13+,wcp16+,wcp2+)</td>
<td>der(13)(t;13;19)(wcp19+,wcp13)+</td>
<td>der(14)(t;14;15)(wcp15+,wcp14+)</td>
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<tr>
<td>der(13)(q13;13)(wcp13+,wcp1+)</td>
<td>der(13)(q13;13)(wcp13+,wcp1+)</td>
<td>der(14)(t;3;14)(wcp14+,wcp3+)</td>
<td>der(15)(t;13;15)(wcp13+,wcp15+)</td>
</tr>
<tr>
<td>der(14)(t;3;14)(wcp14+,wcp3+)</td>
<td>der(14)(t;3;14)(wcp14+,wcp3+)</td>
<td>der(15)(t;15;20)(wcp20+,wcp15+)</td>
<td>der(15)(t;4;15)(wcp15+,wcp15+)</td>
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<tr>
<td>del(15)(t;15;20)(wcp20+,wcp15+)</td>
<td>del(15)(t;15;20)(wcp20+,wcp15+)</td>
<td>i(15)(p10)(wcp15+)</td>
<td>der(15)(t;4;15)(wcp15+,wcp15+)</td>
</tr>
<tr>
<td>der(16)(t;16;16)(wcp16+,wcp6+)</td>
<td>der(16)(t;16;16)(wcp16+,wcp6+)</td>
<td>der(16)(t;16;16)(wcp16+,wcp6+)</td>
<td>add(15)(wcp8+,wcp15+,wcp1+,wcp10+)</td>
</tr>
<tr>
<td>der(18)(t;16;16)(wcp16+,wcp6+)</td>
<td>der(18)(t;16;16)(wcp16+,wcp6+)</td>
<td>der(18)(t;16;16)(wcp16+,wcp6+)</td>
<td>del(Y)(t;1;20)(wcpY+,wcp20+)</td>
</tr>
</tbody>
</table>

Simple deletions and reciprocal translocations have been described using standard ISCN nomenclature. Derivative and complex rearrangements are described from p to q arm using wcp (whole chromosome paint) nomenclature for each chromosome. Where their origin is indeterminable, the prefix add was used.

* Abnormalities not common between LNCaP and LNCaP.FCG.
* Consistent rearrangements observed in hormone-resistant cell lines.
* Consistent aberrations shared in three or more cell lines.

Involving chromosomes 5 (q35) and 10 (q24.2) [38]. M-FISH analysis was not able to detect this abnormality, possibly because of the small telomeric region involved in the translocation or because the abnormality was not present in the population of cells analyzed. LNCaP and LNCaP.FCG are both derived from the same patient but possess slightly different physiologic characteristics. LNCaP.FCG is a subclone of the parent cell line LNCaP with the characteristics of fast growth in vitro. The data presented here suggests that these differing phenotypic variations may be a result of underlying chromosome abnormalities. As expected, most aberrations detected in LNCaP were also detected in LNCaP.FCG, supporting the identical origin of these cell lines. However, they did contain rearrangements that were apparently cell line specific. The del(13)(q14) observed in LNCaP supports previous comparative genomic hybridization (CGH) data demonstrating loss of 13q14–qter [9]. Because M-FISH was restricted to 10 metaphases, it is possible that these rearrangements existed at low levels. A larger study of these rearrangements existed at low levels. A larger study of
16p, and Yq. PC3 exhibited abnormalities of chromosome 1, 2q, 4, 4, 5, 6q, 8, 10, 11, 12q, 14, and 15. Data from both these cell lines is in accordance with previously published CGH [9] and SKY data [29,30]. PC3 contained several fragments of chromosome 10 throughout the genome, possible reflecting amplification of these regions or inappropriate regulation of genes close to breakpoints. Loss of 10q has been previously reported in prostate cancer [40–44] as well as endometrial adenocarcinoma, glioma, melanoma, lymphoma, and renal cell carcinoma [45–52]. PC3 also contained chromosome 8 material scattered across the genome, possibly reflecting amplification of 8q24, which has been found to be one of the most common genetic alterations in hormone refractory and metastatic prostate cancer [53,54]. Increased copy numbers of chromosomes 8 and 8q24 have been associated with advanced stage and poor prognosis in prostate cancer [55,56], which may imply regions of tumor suppression or oncogenic activity at these loci.

Both hormone-refractory cell lines, DU145 and PC3, exhibited a higher number of chromosome rearrangements than the hormone-responsive cell lines. This increase in genetic alteration in hormone-refractory prostate cancer has been previously described using CGH [9,53,54,57,58]. These clonal rearrangements may reflect underlying genetic mechanisms that are associated with androgen-independent growth. This high frequency of rearrangement may be a result of increased genetic instability caused by deficient cell cycle control or DNA repair. Although both androgen-resistant cell lines exhibited several common clonal rearrangements, the breakpoints of these rearrangements could not be ascertained. Two alterations shared by the hormone-unresponsive cell lines that could be accurately evaluated were del(4)(p14) and del(6)(q21), which may reflect deletions of genes involved in hormone receptor production or cellular response to hormones.

No rearrangements of chromosomes 7, 17, 18, 22, and X were observed in any cell line. Several common chromosome alterations were observed in three of the four cell lines, suggesting that these rearrangements may be strongly associated with prostate tumorigenesis. Possibly of the most interest is the i(5p) observed in LNCaP.FCG, DU145 and PC3. The i(5p) has been previously described in DU145

Fig. 7. Abnormalities detected by three individual studies in prostate carcinoma cell lines, LNCaP, DU145, and PC3, using multicolor FISH technologies. Each color reflect the default M-FISH colors used in this study. The chromosomes that each marker is derived from are specified next to each chromosome. (Ai), (Bi), and (Ci) were detected in all three studies; (Aii), (Bii), and (Civ) were detected in all studies but may have been characterized differently; (Biii), (Ciii), and (Cvi) were detected in two of the three studies; and (Ci) and (Cii) were detected in one or more studies but characterized differently.
[19] and PC3 [59], but not in LNCaP.FCG. Previous cytogenetic analysis of LNCaP showed no abnormality of chromosome 5, although aberrations of this chromosome have been reported in other prostate cancer cell lines [39]. Most alterations of chromosome 5 result in partial or complete loss of 5q and may reflect the position of one or more tumor suppressor genes involved in human prostate cancer development. An apparently similar translocation involving chromosomes 1 and 15 was detected in LNCaP, LNCaP.FCG, and PC3, although it is not possible at this stage to determine whether the breakpoints are identical. DU145 also contained an abnormal chromosome 1, der(1)t(1;4), with breakpoints in a similar region of the p arm of chromosome 1.

To date, three groups have characterized these prostate cell lines using multicolor fluorescence technologies, two using spectral karyotyping [29,30] and the work presented here with M-FISH. LNCaP, the androgen-dependent cell line, showed a very similar karyotype in all three studies, suggesting that this cell line is karyotypically stable. Not only did all three studies observe consistent rearrangements in the prostate cell lines, DU145 and PC3, but they also showed a high frequency of single clonal abnormalities specific to a single study. This may reflect high levels of genetic instability in these cell lines, allowing consequent in vitro karyotypic evolution in a specific laboratory or cell line supplier. The number of passages that each of the cell lines had undergone before harvest was only available from one of the previously published reports [30]. Although the use of passage is a relatively inaccurate method to assess in vitro cell growth, the number of passages used in each study were similar. This suggests that it is genetic instability induced by the tumor-related genetic change, rather than by increased passage, that has lead to the chromosome differences observed between the three studies.

The three studies discussed have permitted more accurate characterization of several markers that were initially observed by G-banding [19,20,35]. As discussed previously, these rearrangements may reflect changes in prostate physiology and hormone sensitivity. This review has also highlighted several other groups of derivative chromosome. First, there are those aberrations that were observed in two studies, which may reflect subclones present in the parent-cell line, that were not sampled for or detected by either one of the other research studies. Each study assessed chromosome rearrangement in 10 metaphase cells, which may allow abnormalities in subclonal populations to go undetected. Second, there are those abnormalities only observed in a single study, which may reflect karyotype evolution in a subpopulation of cells specific to the cell line used in that study. Both M-FISH and SKY allow the entire genome to be screened for chromosome rearrangement in a single reaction. M-FISH uses a combinatorial filter-based approach, whereas SKY analysis uses a sensitive interferometer to differentiate between each specifically painted chromosome. Several derivative chromosomes detected in these three studies may be incorrectly classified using this technology, and hence may demonstrate limitations in the use of these techniques in the characterization of established cell lines. Of particular interest is the large derivative chromosome 10 (Fig. 7, C iv) detected in each study, but classified differently. It seems likely that this chromosome has been incorrectly characterized in two or more of these studies, because it is unlikely that such a complex chromosome evolved independently in different laboratories. In the present study, conventional chromosome painting was used to confirm the presence of chromosome 1 material in this marker. Both M-FISH and SKY analysis have become very popular technologies in the characterization of established cell lines and primary tumors. Although the work discussed here represents a relatively small study, it does suggest that data produced with these new techniques should always be confirmed with conventional chromosome painting, particularly in complex karyotypes. It would also be useful for a larger study to compare these technologies more accurately, without such variation as cell line source or separate cell culture lineage.

In the present study, we describe a translocation between chromosome 1 and 15 in both LNCaP and PC3. Both spectral karyotyping studies also observed this translocation in LNCaP, and one of the studies [30] observed a similar translocation in PC3 (Fig. 7, C ii). Using these technologies, it is impossible to elucidate exact breakpoints, although they appear to be in similar chromosomal regions. Interestingly, in DU145, all three studies also described a translocation between chromosomes 1 and 4 (Fig. 7, B ii). M-FISH analysis suggests the breakpoint on chromosome 1 is in a similar euchromatic region of the p arm. Previously published data suggests that the breakpoint may be in either the euchromatin [29] or the heterochromatin [30] of chromosome 1. It is unknown whether the breakpoints on the p arm of chromosome 1 are the same in these two translocations. A FISH study using unique sequence probes that map along this region of chromosome 1 would delineate the breakpoints more accurately.

In summary, a number of rearrangements have been identified in LNCaP, LNCaP.FCG, DU145, and PC3. Several of these rearrangements are novel. The M-FISH data presented here demonstrate several similarities to the previously described SKY analysis. Differences between these studies may reflect clonal variations or variations in the accuracy of the technologies. Consistent aberrations have been seen between cell lines, suggesting common initiating events in the development of prostate cancer. Cell line-specific abnormalities have been discovered that may be associated with the differences in the biologic properties and in androgen-independent growth. Further in-depth analysis of the common breakpoints associated with different phenotypes may elucidate critical genes involved in prostate tumorigenesis.

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