Identification of a high frequency of chromosomal rearrangements in the centromeric regions of prostate cancer cell lines by sequential Giemsa-banding and spectral karyotyping.

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ABSTRACT

BACKGROUND: Currently, prostate cancer (CaP) cytogenetics is not well defined, largely due to technical difficulties in obtaining primary tumor metaphases.

METHODS AND RESULTS: We examined three CaP cell lines (LNCaP, DU145, PC-3) using sequential Giemsa-banding and spectral karyotyping (SKY) to search for a common structural aberration or translocation breakpoints. No consistent rearrangement common to all three cell lines was detected. A clustering of centromeric translocation breakpoints was detected in chromosomes 4, 5, 6, 8, 11, 12, 14, and 15 in DU145 and PC-3. Both these lines were found to have karyotypes with a greater level of complexity than LNCaP.

CONCLUSIONS: The large number of structural aberrations present in DU145 and PC-3 implicate an underlying chromosomal instability and subsequent accumulation of cytogenetic alterations that confer a selective growth advantage. The high frequency of centromeric rearrangements in these lines indicates a potential role for mitotic irregularities associated with the centromere in CaP tumorigenesis.

INTRODUCTION

Prostate cancer (CaP) is the leading cancer incidence and the second most common cause of cancer mortality in men in North America [1]. However, our understanding of the molecular genetic changes that underlie the progression of this disease remains at an early stage. Since it is well known that chromosomal translocations can lead to disruption of tumor suppressor gene function as well as activation of proto-oncogenes [2], identification of such rearrangements is a critical step towards understanding the development of this tumor. There are numerous examples in leukemias and soft tissue sarcomas where detailed cytogenetic analysis has identified consistent chromosomal aberrations leading to the isolation of causative genes [3, 4].

The cytogenetics of solid tumors has been hampered in comparison to hematological malignancies due to poor success in short-term culture, and inadequate representative metaphase spreads of good quality. Prostate cancer has been particularly problematic in this regard, since the tumor is slow-growing with a low mitotic index, and consequently there is a greater risk that normal stromal cell overgrowth will occur within a short duration of culture [5-7]. To circumvent some of these difficulties a variety of different tissue culture protocols have been implemented, including selection in favor of tumor cells and against normal cell overgrowth [8-12]. Using such procedures a number of consistent cytogenetic alterations have been identified generally affecting chromosomes 7, 8, 10, and Y [5, 13-15]. Nevertheless, no consistent structural chromosome aberrations have been identified in CaP and it remains conceivable that technical limitations on the quality of the cytogenetic preparations derived from primary tumor material have precluded identification of causative structural chromosomal alterations in this tumor.

In light of these difficulties, the detailed study of CaP cell lines has provided some insight into the progression of the disease and classical Giemsa-banding (G-banding) analysis of three of the commonly studied CaP cell lines, LNCaP, DU145, and PC-3 has provided useful information on the extent of cytogenetic change and karyotype evolution [16-19]. Cytogenetic analysis of LNCaP using standard G-banding methods revealed a relatively simple karyotype involving one

reciprocal and one nonreciprocal translocation, and three deletions [17]. The t(6;16)(p21;q22) translocation was recently shown to result in the production of a novel chimeric fusion transcript, Tpc-Hpr, that is thought to interfere with normal ribosomal function [20]. This translocation appears to be an isolated finding, as neither DU145 nor PC-3 has this rearrangement. However, both these lines have highly aberrant karyotypes in comparison to LNCaP and show many marker chromosomes and complex rearrangements with compound regions that cannot be identified by G-banding [18, 19]. Although the use of chromosome painting has helped in the identification of some of the complex marker chromosomes in these two cell lines, the origin(s) of many of these highly abnormal chromosomes remains unknown [21].

In order to more accurately define the karyotypes of these three cell lines, we have used the new technique of spectral karyotyping (SKY) in combination with G-banding. SKY is a "24-color" fluorescence *in situ* hybridization (FISH) approach that uniquely identifies each chromosome based on its specific spectral color composition [22], and the technique allows for the unambiguous identification of individual chromosome fragments involved in complex chromosomal rearrangements and marker chromosomes. By analyzing SKY results in conjunction with the findings from conventional G-banding using the same metaphase spread, it is possible to identify individual regions of specific chromosomes and accurately define all structural rearrangements present.

In this study we have applied sequential G-banding and SKY to the three CaP cell lines LNCaP, DU145 and PC-3 in order to: (1) search for all previously unidentified structural chromosomal rearrangements in each cell line; (2) determine if there are any consistent rearrangements, or cryptic or 'masked' chromosomal changes common to all three cell lines; and (3) fully characterize the more complex chromosomal rearrangements present in DU145 and PC-3.

MATERIALS AND METHODS

Tissue Culture and Cytogenetic Preparations

LNCaP (CRL-1740), DU145 (HTB-81), and PC-3 (CRL-1435) were obtained from the American Type Culture Collection (ATCC, Rockville, MD). LNCaP, an androgen-dependent cell line originating from a lymph node metastasis [16, 23], was grown in RPMI 1640 with 2 mM L-glutamine, 1.5 g/L sodium bicarbonate, 4.5 g/L glucose, 10 mM HEPES, 1.0 mM sodium pyruvate, and 10% fetal bovine serum. DU145, an androgen-independent cell line obtained from a metastasis to the bone [18], was grown in F15K Minimum essential medium with 1.5 g/L sodium bicarbonate and 10% fetal bovine serum. PC-3, also an androgen-independent cell line and originated from a brain metastasis [19], was grown in Ham's F12K with 2 mM L-glutamine, 1.5 g/L sodium bicarbonate, and 10% fetal bovine serum.

Cytogenetic preparations of LNCaP (passage 23), DU145 (passage 83), and PC-3 (passage 38) were made according to standard protocols [24] using colcemid and KCl hypotonic treatment. The slides were karyotyped following a standard G-banding protocol [24], and images of ten metaphases in which there was minimal chromosome overlap, long chromosome length, little or no cytoplasm, and high banding resolution were selected for detailed analysis. Microscope co-ordinates of all digitized G-banded preparations were recorded, so that the metaphase cells analyzed by G-banding could be analyzed concurrently by SKY methods.

Spectral Karyotyping (SKY)

The SKY™ KIT probe cocktail from Applied Spectral Imaging (ASI, Carlsbad, CA) was hybridized to metaphase spreads from each CaP cell line according to standard protocols [22, 25, 26] and the manufacturer's instructions (ASI, Carlsbad, CA). Briefly, after destaining the G-banded slides with methanol for 10 minutes, the slides were rehydrated in a descending ethyl alcohol series (100%, 90%, 70%), and fixed with 1% formaldehyde in 50mM $MgCl_2$/phosphate buffer solution for 10 minutes. The slides were then dehydrated using an ascending ethyl alcohol

series, and denatured for 30-45 seconds in 70% formamide/2XSSC at 75°C. The SKY probe was denatured for 7 minutes at 75°C, reannealed at 37°C for 1 hour, placed on the slide and covered with a glass coverslip. The coverslip was sealed with rubber cement and the slides placed in a damp container in a 37°C incubator. After hybridizing overnight, the post-hybridization washes were performed per manufacturer's instructions (ASI, Carlsbad, CA).

The metaphase images were captured using an SD 200 spectral bio-imaging system (ASI Ltd., MigdalHaemek, Israel) attached to a Zeiss microscope (Axioplan 2) and stored on a SKY image-capture workstation. The images were analyzed using the SKYView software version 1.2 (ASI, Carlsbad, CA), which resolves individual fluorochrome spectra by Fourier spectroscopy and distinguishes the spectral signatures for each chromosome to provide a unique pseudocolour for each chromosome (classified image). G-banding and SKY analyses were performed sequentially on each of the three cell lines with the same ten metaphase images captured for G-banding also analyzed by SKY. Because of the presence of nonclonal changes in DU145 and PC-3, composite karyotype descriptions were made for these two cell lines.

The determination of the position of translocation breakpoints was performed by aligning the G-banding pattern for each rearranged chromosome with its respective SKY pseudocolor classified image, and mapping each translocation boundary with respect to the associated G-banded chromosomal interval and the ISCN designation [27] for the band locations where breakage and rearrangement has occurred.

RESULTS

Sequential G-banding and SKY analysis of LNCaP cells on a metaphase-by-metaphase basis confirmed the bimodal diploid and tetraploid chromosome number [17]. Overall, LNCaP demonstrated a consistent karyotype, with few nonclonal changes (incidental gains/losses and/or structural rearrangements not contributing to the karyotype) per metaphase (Table 1). Six of the seven previously reported marker chromosomes [17] were confirmed by G-banding and SKY analyses in 10/10 metaphases (Figure 1). Marker 7 (an interstitial deletion of 13q21.1), was detected in 9/10 metaphases but was absent in the metaphase shown in Figure 1. The level of resolution afforded by the current sensitivity of the SKY system enabled identification of a cryptic or 'hidden' novel rearrangement in LNCaP. Markers 3 and 6, previously identified by G-banding to be involved in a nonreciprocal translocation of a fragment of 6p onto 16q [17], were instead found by SKY to be involved in a reciprocal t(6;16). When normalized to a diploid chromosome number, LNCaP cells were found to have 9 structural aberrations per metaphase. For example: the reciprocal t(1;15)x2 counted as four aberrations, the der(6)t(4;6)x2 as two aberrations, and the del(2) as one aberration; numerical changes such as the loss of chromosome 2 were not included in the count. G-banding and SKY analysis of LNCaP metaphase cells revealed few structural aberrations per metaphase, indicating that the karyotype was relatively simple.

As previously reported [18, 21], DU145 was observed to have a hypotriploid chromosome number with more complex karyotypic changes than LNCaP, showing approximately 18 aberrations per diploid cell (Figure 2). Chromosomal loss in DU145 was more common than gain, with losses of whole chromosomes 2, 3, 4, 13, 16, 19, 20, 21, 22, and X, and partial losses of 5q, 9p and 11q; and gains of chromosome 18 and derivative chromosomes 5 and 9 (Table 1). Structural chromosomal changes of interest were the t(5;21) and t(4;6) translocations that were not detected by G-banding analyses, but were easily identified by SKY. Other translocations, such as the t(1;4), t(Y;20), t(2;13), t(6;16) and t(9;11), were recognized as abnormal derivative chromosomes 1, Y, 2, 6 and 9 by G-banding analysis, but required SKY analysis for identification of the involved partner

chromosomes (Table 1). The previously unidentified minute chromosomes observed by Stone et al. [18] were determined by SKY analysis to be derived from chromosome 5. The sequential analysis of DU145 by G-banding and SKY allowed the identification of 27 structural breakpoints of which 14 involved centromeric or pericentromeric regions. More than half of the chromosomes in the DU145 genome showed rearrangements involving centromeric breaks. DU145 demonstrated more nonclonal changes per metaphase than either LNCaP or PC-3.

PC-3 cells were also observed to be hypotriploid and demonstrated more karyotypic abnormalities than either LNCaP or DU145 cells, with approximately 34 aberrations per diploid cell. Almost every chromosome in this cell line had either structural or numerical abnormalities (Figure 3) with chromosomal loss being more prevalent than gain. PC-3 exhibited losses of whole chromosomes 3, 5, 8, 9, 10, 15, 16, 17, 19, and 22, and partial loss of chromosomes 6q, 8p, and 17p. In addition, whole chromosomal gains of 1, 7, 11, 14, 20, and 21 were observed, and an additional gain of chromosome 14 was observed in 4/10 metaphases (Table 1). Seven complex rearrangements involving more than 2 chromosome partners were characterized in this cell line. Sequential G-banding and SKY analysis of PC-3 allowed the identification of 37 structural breakpoints of which 8 involved centromeric or pericentromeric regions. Many of the structural rearrangements were paired, suggesting that these changes occurred in a diploid progenitor that subsequently underwent tetraploidization. The isochromosome 5p, previously reported by Bernadino et al. [21], was also identified by SKY in both DU145 and PC-3.

In both DU145 and PC-3, marker chromosomes which had been partially characterized by classical banding and chromosome painting approaches were more fully characterized by sequential G-banding and SKY. Listed in Table 2 are several examples of marker chromosomes whose identities previously reported by Bernardino et al. [21] have been further defined by sequential G-banding and SKY analyses.

The composite G-banding and SKY karyotype results for the three CaP cell lines shown in Table 1 demonstrate an increasing complexity of chromosomal aberrations, with LNCaP having the simplest pattern of chromosomal change, followed by DU145 with intermediate complexity, and

PC-3 as the more complex line. While no consistent rearrangement or common chromosomal aberration was detected using the increased sensitivity afforded by SKY, examination of DU145 and PC-3 revealed eight chromosomal rearrangements involving breakage within the centromeric regions of chromosomes 4, 5, 6, 8, 11, 12, 14, and 15 (Figure 4). Furthermore, DU145 was found to have involvement of eight additional centromeric or pericentromeric rearrangements on chromosomes 1, 2, 7, 10, 16, 19, 20, and 21; PC-3 had only one additional involvement on chromosome X.

DISCUSSION

Obtaining a detailed characterization of chromosomal abnormalities in solid tumors by classical cytogenetics has been limited by difficulties in both culturing fresh tumor tissue and in obtaining good quality representative banded metaphase preparations. The use of tumor cell lines has provided an alternative resource for studying cytogenetic changes in greater depth, and the recent development of SKY has significantly enhanced the ability to detect and comprehensively identify the structural aberrations present in any cell line [28]. However, SKY analysis as a single method of chromosome identification has significant limitations. For example, the current SKY probe kit does not permit detection of intrachromosomal dosage changes or interstitial structural rearrangements. In addition, SKY classification does not provide information on the region of the abnormal chromosome involved in the rearrangement. We have therefore used a sequential approach of G-banding followed by SKY to examine the identity of all chromosomal aberrations present in the three CaP cell lines, LNCaP, DU145 and PC-3. Similar sequential methods were recently reported to identify the origins an unusual marker chromosome in a leukemia [29].

The advantages of the sequential approach of G-banding and SKY are evident in genomes demonstrating increased karyotypic complexity, such as DU145 and PC-3. G-banding data for both these cell lines [18] was unable to fully characterize the observed chromosomal aberrations. The use of pair-wise combinations of chromosome paints provided more information on the chromosomes involved in rearrangements [21]. For example, while Stone et al. identified a marker Y chromosome by G-banding, the partner chromosome was unidentifiable by this method [18]. Bernardino et al. [21] used pair-wise combinations of chromosome-painting FISH experiments to resolve the identity of this marker chromosome as a der(Y)t(Y;20)(q12;?). Despite these advantages, karyotyping by pair-wise chromosome painting is cumbersome and limited by the number of potential combinations of chromosomal rearrangements found in derivative chromosomes. Combined G-banding and SKY has overcome these limitations and permitted

further characterization of novel rearrangements and more precise definition of previous rearrangements in DU145 and PC-3 (Table 2).

The chromosomal stability of the karyotypes present in each cell line is also a consideration when comparing cytogenetic findings ascertained using different sources of the same cell line and at different cell passage number. In our analysis, sequential G-banding and SKY revealed a cryptic novel translocation of a small fragment of 16q onto 6p, but did not reveal any additional chromosomal changes in LNCaP in comparison to previous G-banding results [17]. However, a study by Ford et al. using whole-chromosome paints [30] detected the nonreciprocal translocation of 10q24 material to two sites on chromosome 5q forming a derivative chromosome 5 that was not present in our analysis. Similarly, a recent SKY analysis of LNCaP cells reviewed by Brothman et al. [31] demonstrated additional chromosomal rearrangements, such as t(15;22) and t(3;11), also not observed in our analysis. Whether these rearrangements in LNCaP are representative clonal changes is unclear. Previous studies have shown that the karyotype of DU145 also varies as a function of passage number. Both Stone et al. and Bernardino et al. found that the DU145 karyotype was stable through 90 passages, and at passage 73 the cells had a near-triploid chromosome number with extensive chromosomal rearrangements [18, 21]. By passage 153, however, DU145 was found to have a near-tetraploid karyotype with an increased number of rearrangements [18, 21]. The karyotype for DU145 (passage 83) reported herein is comparable with that reported by Stone et al. and Bernardino et al. below passage 90 [18, 21]. In contrast to DU145, PC-3 is believed to be a karyotypically stable cell line [19]. This is supported by the study of Camby et al. [32] that showed PC-3 to be more hormone-sensitive and to maintain a higher degree of differentiation than DU145. Kaighn et al. described PC-3 as 100% aneuploid with complete losses of chromosomes 1, 2, 3, 5, 15, and Y; and the presence of at least ten marker chromosomes per metaphase spread [19], a finding confirmed by the present study. The prevalence of chromosomal losses over gains seen in both DU145 and PC-3 is supported by recent comparative genomic hybridization (CGH) findings [33].

Our results for the CaP cell lines showed that in terms of the karyotypic complexity of rearrangements, LNCaP < DU145 < PC-3, with approximately 9, 18, and 34 structural aberrations per diploid cell, respectively (Table 1). This finding is in agreement with the suggestion by Nupponen et al. that DU145 and PC-3 represent the more advanced, androgen-independent CaP disease state while LNCaP resembles more closely primary CaP disease [33]. This would support the concept that the stepwise progression to a more advanced disease state, as modeled by DU145 and PC-3, involves an accumulation of chromosomal alterations that may confer selective growth advantages.

Sequential G-banding and SKY analyses demonstrated that there was no common chromosomal rearrangement or common translocation breakpoint present in all three CaP cell lines. When comparing breakpoint regions of DU145 and PC-3, the most common shared feature was involvement of the centromeric regions of chromosomes 4, 5, 6, 8, 11, 12, 14, and 15 in structural chromosomal aberrations. In contrast, LNCaP was observed to have only one centromeric rearrangement on chromosome 10. The high involvement of the centromeric regions in DU145 and PC-3 is of interest because the centromere plays an essential role in maintaining diploidy [34]. The greater frequency of aberrations at centromeric and pericentromeric regions in DU145 than PC-3 may be of importance given the increased instability observed in DU145 through passaging [18, 21]. Only monocentric chromosomes were observed in all three cell lines suggesting orderly chromosome separation, which is not seen in cells containing ring, dicentric and multicentric chromosome structures [34]. Normally the centromere is the last chromosomal segment that is replicated in monocentric mammalian chromosomes during cell division [35]; however, premature centromere separation could lead to the type of aneuploidy [34] observed in DU145 and PC-3. The significant involvement of centromeric breakpoints may reflect the high degree of chromosomal misdivision and sister-chromatid exchange, or increased instability of the pericentromeric regions during mitosis [34, 36]. There is an increasing interest in understanding the role of the kinetochore in normal and abnormal mitosis [37, 38] and its relationship to the acquisition of centromeric aberrations and aneusomies in cancer cells [39, 40].

While the use of tumor cell lines has provided an alternative resource for studying cytogenetic changes in carcinomas that ordinarily would present difficulties in tissue culture, the question is raised as to whether the cytogenetics remain representative of primary tumors. Given the slow onset pathology of CaP, however, it may be surmised that the initiating event(s) may not be a single genetic alteration, but instead due to aberrations in cell division. In this regard, the accumulation of multiple genetic aberrations during CaP progression may be downstream effects which confer selective growth advantages. The observed alterations at the centromeric regions support this view and suggest that amidst the other chromosomal aberrations within each cell line, the initial tumorigenic events are not lost in the cell lines studied.

In summary, sequential G-banding and SKY is an effective FISH-based whole-genome screening technique that significantly improves the ability to identify cryptic and complex chromosomal rearrangements in tumor cells. Using this approach we have confirmed and more precisely defined the karyotypes of three CaP cell lines, identifying a cryptic novel rearrangement in LNCaP and resolving previously unknown marker chromosomes and complex rearrangements in the more complicated DU145 and PC-3 genomes. No consistent translocation breakpoint, suggestive of a common structural rearrangement in all three cell lines was observed; however, centromeric breakpoints were demonstrated to be the most frequent shared feature between DU145 and PC-3. Our results imply that karyotypically, LNCaP may be less advanced than DU145 and PC-3. This observation is in agreement with the multistep model of accumulated hits in CaP tumorigenesis and suggests an increasing importance in understanding the role of the centromere in CaP tumorigenesis.

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REFERENCES

1. Haas GP, Sakr WA: Epidemiology of prostate cancer. CA Cancer J Clin 1997;47:273-287
2. Weinberg R: How cancer arises. Scientific American 1996;275:62-70
3. Rowley JD: The critical role of chromosome translocations in human leukemias. Annu Rev Genet 1998;32:495-519
4. Ladanyi M: The emerging molecular genetics of sarcoma translocations. Diagn Mol Pathol 1995;4:162-173
5. Webb HD, Hawkins AL, Griffin CA: Cytogenetic abnormalities are frequent in uncultured prostate cancer cells. Cancer Genetics & Cytogenetics 1996;88:126-132
6. Konig JJ, Teubel W, van Dongen JW, Hagemeijer A, Romijn JC, Schroder FH: Tissue culture loss of aneuploid cells from carcinomas of the prostate. Genes, Chromosomes & Cancer 1993;8:22-27
7. Ketter R, Zwergel T, Romanakis K, et al.: Selection toward diploid cells in prostatic carcinoma derived cell cultures. Prostate 1996;28:364-371
8. Chopra DP, Sarkar FH, Grignon DJ, Sakr WA, Mohammed A, Waghray A: Growth of human nondiploid primary prostate tumor epithelial cells in vitro. Cancer Research 1997;57:3688-3692
9. Limon J, Lundgren R, Elfving P, et al.: An improved technique for short-term culturing of human prostatic adenocarcinoma tissue for cytogenetic analysis. Cancer Genetics & Cytogenetics 1990;46:191-199
10. Peehl DM, Sellers RG, Wong ST: Defined medium for normal adult human prostatic stromal cells. In Vitro Cell Dev Biol Anim 1998;34:555-560
11. Szucs S, Zitzelsberger H, Breul J, Bauchinger M, Hofler H: Two-phase short-term culture method for cytogenetic investigations from human prostate carcinoma. Prostate 1994;25:225-235

12. Zwergel T, Kakirman H, Schorr H, Wullich B, Unteregger G: A new serial transfer explant cell culture system for human prostatic cancer tissues preventing selection toward diploid cells. Cancer Genet Cytogenet 1998;101:16-23

13. Arps S, Rodewald A, Schmalenberger B, Carl P, Bressel M, Kastendieck H: Cytogenetic survey of 32 cancers of the prostate. Cancer Genetics & Cytogenetics 1993;66:93-99

14. Micale MA, Sanford JS, Powell IJ, Sakr WA, Wolman SR: Defining the extent and nature of cytogenetic events in prostatic adenocarcinoma: paraffin FISH vs. metaphase analysis. Cancer Genetics & Cytogenetics 1993;69:7-12

15. Zitzelsberger H, Szucs S, Robens E, Weier HU, Hofler H, Bauchinger M: Combined cytogenetic and molecular genetic analyses of fifty-nine untreated human prostate carcinomas. Cancer Genetics & Cytogenetics 1996;90:37-44

16. Horoszewicz JS, Leong SS, Kawinski E, et al.: LNCaP model of human prostatic carcinoma. Cancer Research 1983;43:1809-1818

17. Gibas Z, Becher R, Kawinski E, Horoszewicz J, Sandberg AA: A high-resolution study of chromosome changes in a human prostatic carcinoma cell line (LNCaP). Cancer Genet Cytogenet 1984;11:399-404

18. Stone KR, Mickey DD, Wunderli H, Mickey GH, Paulson DF: Isolation of a human prostate carcinoma cell line (DU 145). International Journal of Cancer 1978;21:274-281

19. Kaighn ME, Narayan KS, Ohnuki Y, Lechner JF, Jones LW: Establishment and characterization of a human prostatic carcinoma cell line (PC-3). Investigative Urology 1979;17:16-23

20. Veronese ML, Bullrich F, Negrini M, Croce CM: The t(6;16)(p21;q22) chromosome translocation in the LNCaP prostate carcinoma cell line results in a tpc/hpr fusion gene. Cancer Research 1996;56:728-732

21. Bernardino J, Bourgeois CA, Muleris M, Dutrillaux AM, Malfoy B, Dutrillaux B: Characterization of chromosome changes in two human prostatic carcinoma cell lines (PC-3 and DU145) using chromosome painting and comparative genomic hybridization. Cancer

Genet Cytogenet 1997;96:123-128

22. Schrock E, du Manoir S, Veldman T, et al.: Multicolor spectral karyotyping of human chromosomes. Science 1996;273:494-497
23. Horoszewicz JS, Leong SS, Chu TM, et al.: The LNCaP cell line--a new model for studies on human prostatic carcinoma. Prog Clin Biol Res 1980;37:115-132
24. Gustashaw KM: Chromosome Stains. In MJ Barch, T Knutsen and JL Spurbeck: The AGT cytogenetics laboratory manual, 3. Lippincott-Raven Publishers, Philadelphia, 1997, pp. 259-324
25. Veldman T, Vignon C, Schrock E, Rowley JD, Ried T: Hidden chromosome abnormalities in haematological malignancies detected by multicolour spectral karyotyping. Nature Genetics 1997;15:406-410
26. Garini Y, Macville M, Manoir S, et al.: Spectral karyotyping. Bioimaging 1996;4:65-72
27. Mitelman F: ISCN (1995): International System for Human Cytogenetic Nomenclature, S. Karger, New York, 1995
28. Padilla-Nash HM, Nash WG, Padilla GM, et al.: Molecular cytogenetic analysis of the bladder carcinoma cell line BK-10 by spectral karyotyping. Genes Chromosomes Cancer 1999;25:53-59
29. Allen RJ, Smith SD, Moldwin RL, et al.: Establishment and characterization of a megakaryoblast cell line with amplification of MLL. Leukemia 1998;12:1119-1127
30. Ford S, Gray IC, Spurr NK: Rearrangement of the long arm of chromosome 10 in the prostate adenocarcinoma cell line LNCaP. Cancer Genet Cytogenet 1998;102:6-11
31. Brothman AR, Maxwell TM, Cui J, Deubler DA, Zhu XL: Chromosomal clues to the development of prostate tumors. Prostate 1999;38:303-312
32. Camby I, Etievant C, Petein M, et al.: Influence of culture media on the morphological differentiation of the PC-3 and DU145 prostatic neoplastic cell lines. Prostate 1994;24:187-196
33. Nupponen NN, Hyytinen ER, Kallioniemi AH, Visakorpi T: Genetic alterations in prostate

cancer cell lines detected by comparative genomic hybridization. Cancer Genet Cytogenet 1998;101:53-57

34. Vig BK, Sternes KL, Paweletz N: Centromere structure and function in neoplasia. Cancer Genet Cytogenet 1989;43:151-178

35. Broccoli D, Paweletz N, Vig BK: Sequence of centromere separation: characterization of multicentric chromosomes in a rat cell line. Chromosoma 1989;98:13-22

36. Kaiser-Rogers K, Rao K: Structural chromosome rearrangements. In SL Gersen and MB Keagle: The principles of clinical cytogenetics, Humana Press Inc., Totowa, 1999, pp. 191-228

37. Chan GK, Schaar BT, Yen TJ: Characterization of the kinetochore binding domain of CENP-E reveals interactions with the kinetochore proteins CENP-F and hBUBR1. J Cell Biol 1998;143:49-63

38. Faulkner NE, Vig B, Echeverri CJ, Wordeman L, Vallee RB: Localization of motor-related proteins and associated complexes to active, but not inactive, centromeres. Hum Mol Genet 1998;7:671-677

39. Cahill DP, da Costa LT, Carson-Walter EB, Kinzler KW, Vogelstein B, Lengauer C: Characterization of MAD2B and other mitotic spindle checkpoint genes. Genomics 1999;58:181-187

40. Sawyer JR, Tricot G, Mattox S, Jagannath S, Barlogie B: Jumping translocations of chromosome 1q in multiple myeloma: evidence for a mechanism involving decondensation of pericentromeric heterochromatin. Blood 1998;91:1732-1741

FIGURE LEGENDS

Figure 1. G-banding and SKY composite karyogram of LNCaP (passage 23). Giemsa-banded metaphase (top left), spectral metaphase (top middle), pseudocolour classification (top right). There are 87 chromosomes in the metaphase spread. The karyogram (bottom) depicts each chromosome by aligning its G-banded (left chromosome) and classified (right chromosome) representations.

Figure 2. G-banding and SKY composite karyogram of DU145 (passage 83). There are 60 chromosomes in the metaphase spread.

Figure 3. G-banding and SKY composite karyogram of PC-3 (passage 38). There are 62 chromosomes in the metaphase spread.

Figure 4. Breakpoint analysis of the three CaP cell lines. Breakpoints found in the CaP cell lines (LNCaP—red; DU145—blue; PC-3—green) are designated as circles to the right of each chromosome ideogram in the centre of the chromosomal interval where the breakpoint occurs. Clustering of centromeric and pericentromeric breakpoints in DU145 and PC-3 are indicated as bars to the left of the ideograms (cyan).

Cell line	Chromosomal Rearrangements	Structural Aberrations per Diploid Cell
LNCaP	86~90,XXYY,t(1;15)(p22;q24)x2,-2,del(2)(p13~23),der(4)t(4;6)(q21;q?15)t(6;10)(q?25;q11)x2, der(6)t(4;6)(q25;q15)x2,t(6;16)(p21.1;q22)x2,del(10)(q24)x2,del(13)(q21.1),[10]	18/2 = 9
DU145	57~62<3n>,X,-X,der(Y)t(Y;20)(q12;?p11)[10],der(1;4)(q10;p10)[9],-2[10],-3[10],-4[9], der(4)t(4;6)(q31;?)[9],i(5)(p10)[10],+der(5)del(5)(p?13)del(5)(q?11)x2[8], +der(5)t(5;21)(p13;q11.2)[10],der(6;16)(p10;q10)[9],der(7;8)(p10;q10)[7],del(9)(p21)[10], +der(9)del(9)(p13)t(9;11)(q22;?)[8],der(10;19)(q10;?p10)[9],del(11)(q23)[10], der(11;12)(q10;q10)[9],-13[10],der(13)t(2;13)(?p11;q33)[10],der(13)t(11;13)(?q23;q33)[10], der(14)t(3;14)(q21;q31)[8],ider(14)(q10)t(3;14)(q21;q31)[2],der(15;20)(q10;q10)x2[10], -16[10],+18[8],der(18)t(14;18)(q13;q21)x2[10],-19[10],-20[10],-21[10],-22[10],[cp10]	27*(2/3) = 18
PC-3	59~64,XX,-Y,+1[7],der(1)t(1;2)(q22;?)t(1;12)(p31;?)t(8;12)(q13;?)[8], der(1)t(1;15)(p22;q15)t(1;2)(q25;?p21)[9],der(2)t(2;15)(p24;q22)t(15;17)(q11;q12)[10], der(2)t(2;8)(p24;q13)x2[10],-3[10],der(3)t(3;10)(q13;?)x2[10],der(4;6)(q10;p10)[9], der(4)t(4;10)(q21;?)x2[10],der(4;12)(q10;q10)[10],-5[10],i(5)(p10)[9],del(6)(q25~26)[10], +7[9],-8[9],del(8)(p21)[10],der(8)t(X;8)(q10;q10)[5], der(8)t(8;15)(q10;q10)t(15;?17)(q26;?)t(3,?17)(q25;?)[3],-9[9],-10[9], der(10)t(3;10)(p14.1;q21)t(4;10)(?;q25)t(4;10)(?;q21)t(4;10)(?;p12)t(4;10)(?;?)t(3;10)(q13.3;?)x2[10], +11[7],der(11)t(2;11)(?;p11)t(2;19)(?;?)t(5;19)(q13;?)[10],der(11;14)(q10;q10)[3], der(12)t(8;12)(q13;q24.3)x2[10],+14[7],+14[4],der(14)t(X;14)(?p22.1;q32)x2[6], der(14)dup(14)(?)t(14;15)(p12;?)t(15;17)(?;?)t(3;17);(q25;?)[2],-15[10], der(15)t(5;15)(q13;p13)[10],der(15)t(15;17)(?;?)t(15;17)(?;q21)t(3;17)(q25;?)[8],-16[10], -17[9],del(17)(p11.2)[10],-19[8],+20[6],+21[10],-22[10],[cp10]	51*(2/3) = 34

Table 1. Karyotype description of LNCaP (passage 23), DU145 (passage 83), and PC-3 (passage 38) by sequential G-banding and SKY, according to the ISCN convention [27]. LNCaP has a clonal karyotype (ten metaphases). In the composite karyotype descriptions for DU145 and PC-3, numbers in brackets refer to the frequency of occurrence of the directly preceding structural/numerical change (out of ten metaphases).

Cell line	Chromosome Painting Results	Identity by G-banding and SKY
DU145	· add(13)(q33)	· der(13)t(2;13)(?p11;q33)
	· add(13)(q33)	· der(13)t(11;13)(?q23;q33)
	· add(5)(p13)	· der(5)t(5;21)(p13;q11.2)
	· 1-5 markers	· eg.: der(5)del(5)(p?13)del(5)(q?11)x2; der(14)t(3;14)(q21;q31)
PC-3	· add(14)(q32)	· der(14)t(X;14)(?p22.1;q32)
	· der(15)t(5;?;15)(q14;?;p12)	· der(15)t(5;15)(q13;p13)
	· der(11)t(5;10;11)(q14;?;p11)	· der(11)t(5;2;11;19)(q13;?;p11;?)
	· hsr(10)(1;3;10)	· der(10)t(3;10;4;10)
	· der(2)t(2;?;8)(p25;?;q21)x2	· der(2)t(2;8)(p24;q13)x2
	· add(2)(p25)	· der(2)t(2;15;17)
	· 2-5 markers	· eg.: der(8)t(X;8)(q10;q10); der(1)t(2;1;12;8)

Table 2. Examples of chromosome rearrangements that were previously identified by chromosome painting [21] experiments (left), and probable identities found by G-banding and SKY analysis (right). See Table 1 for correct ISCN classifications of listed results.

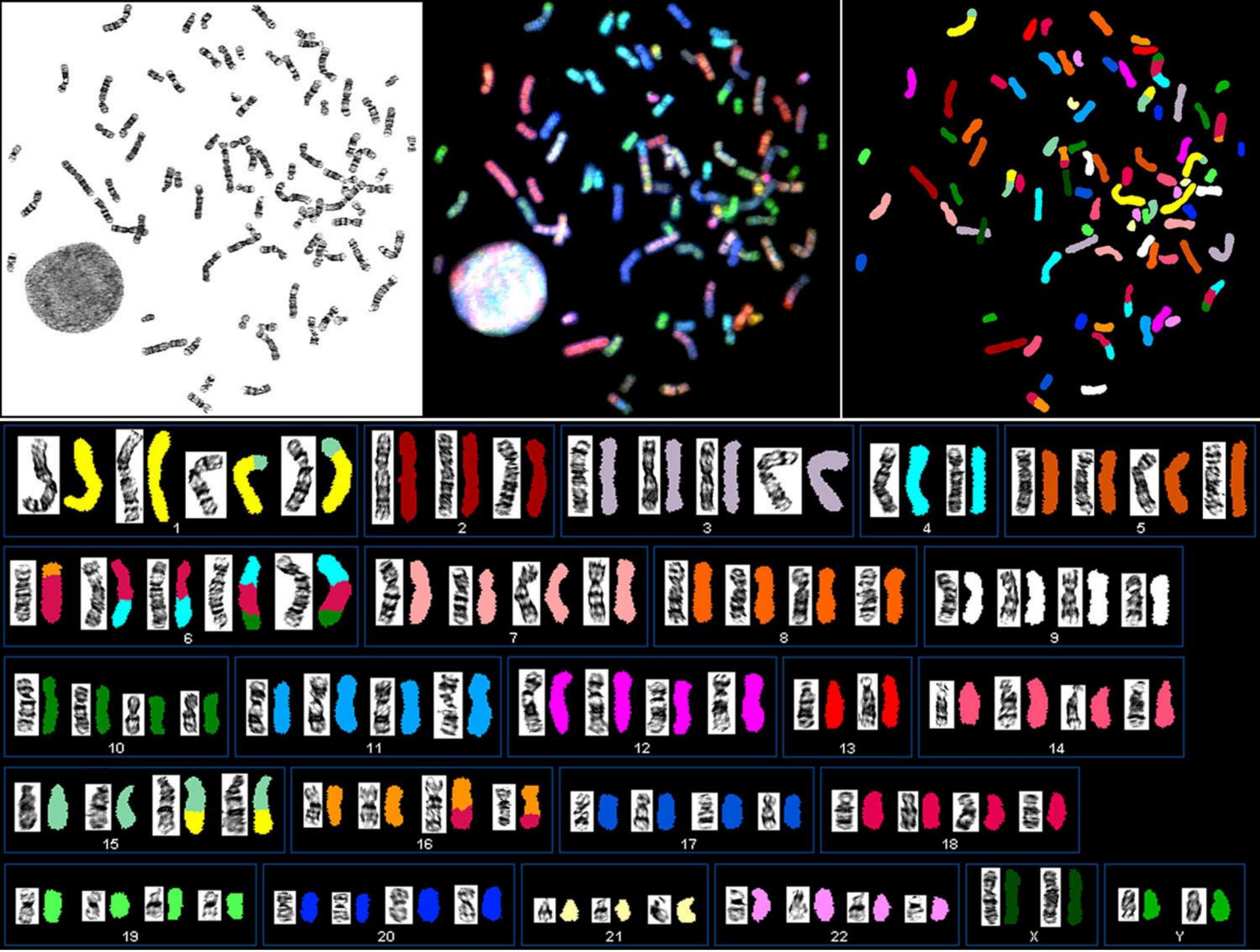

Figure 1 - G-banding and SKY composite of LNCaP (passage 23).

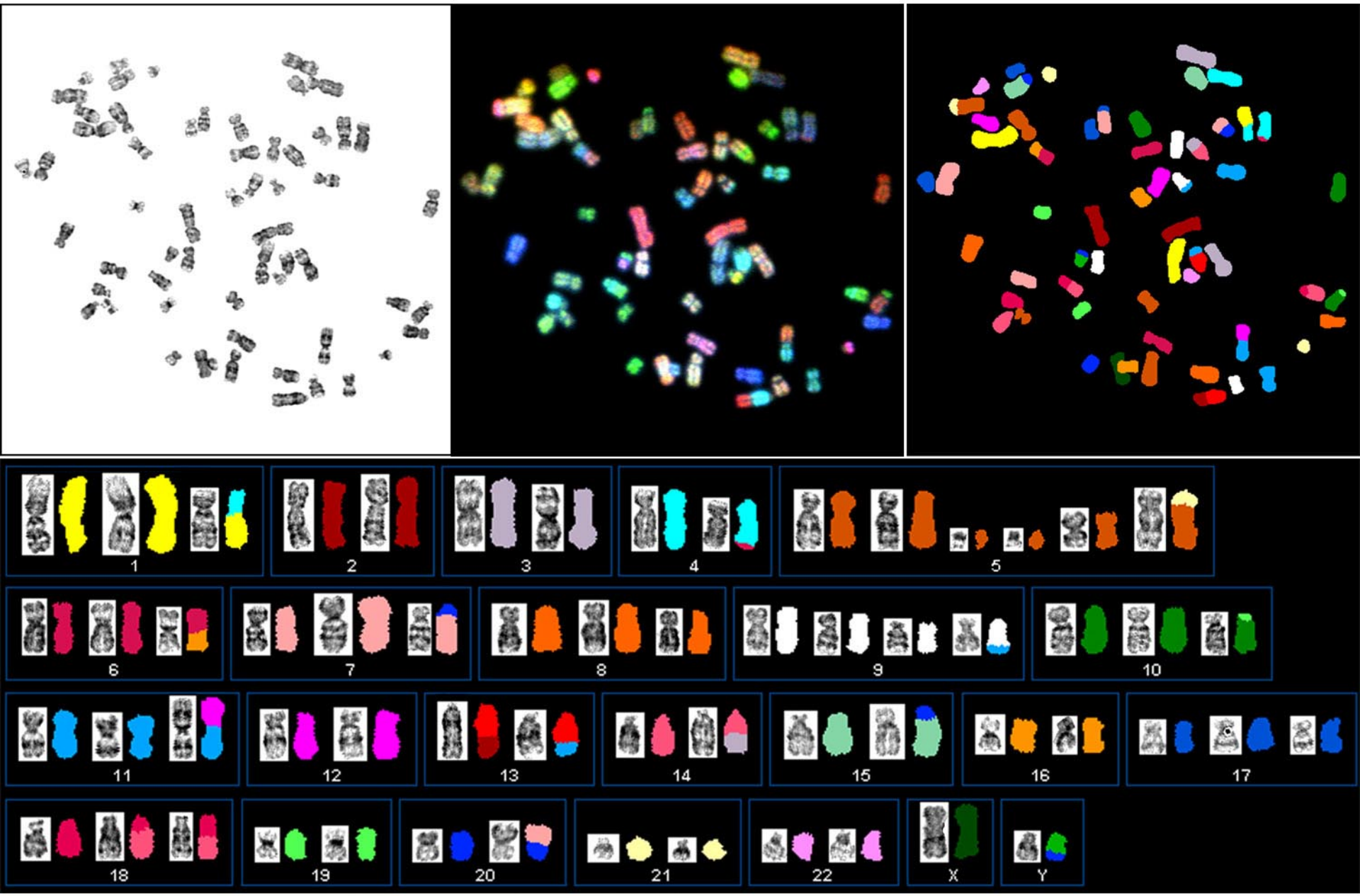

Figure 2 - G-banding and SKY composite of DU145 (passage 83).

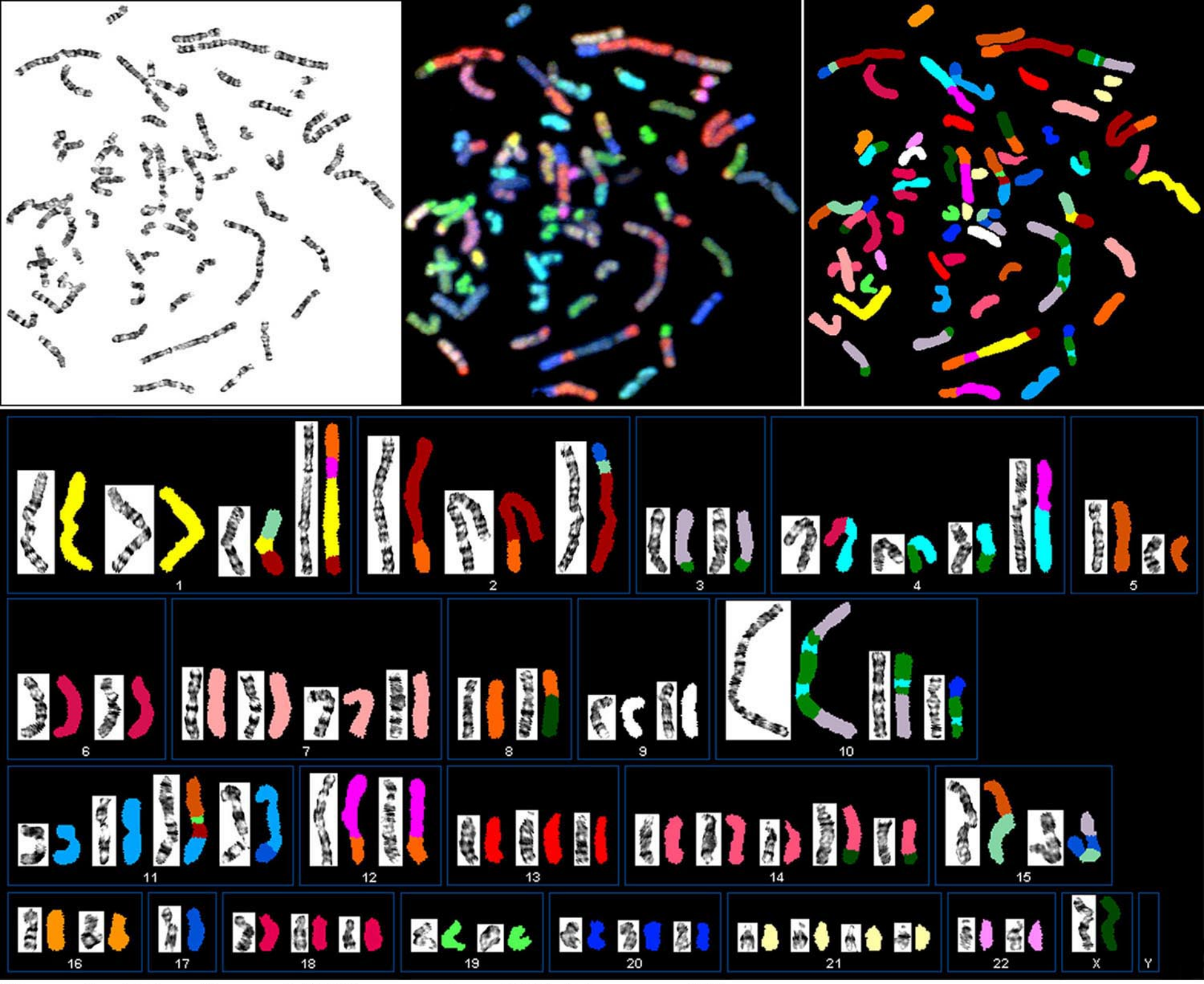

Figure 3 - G-banding and SKY composite of PC-3 (passage 38).

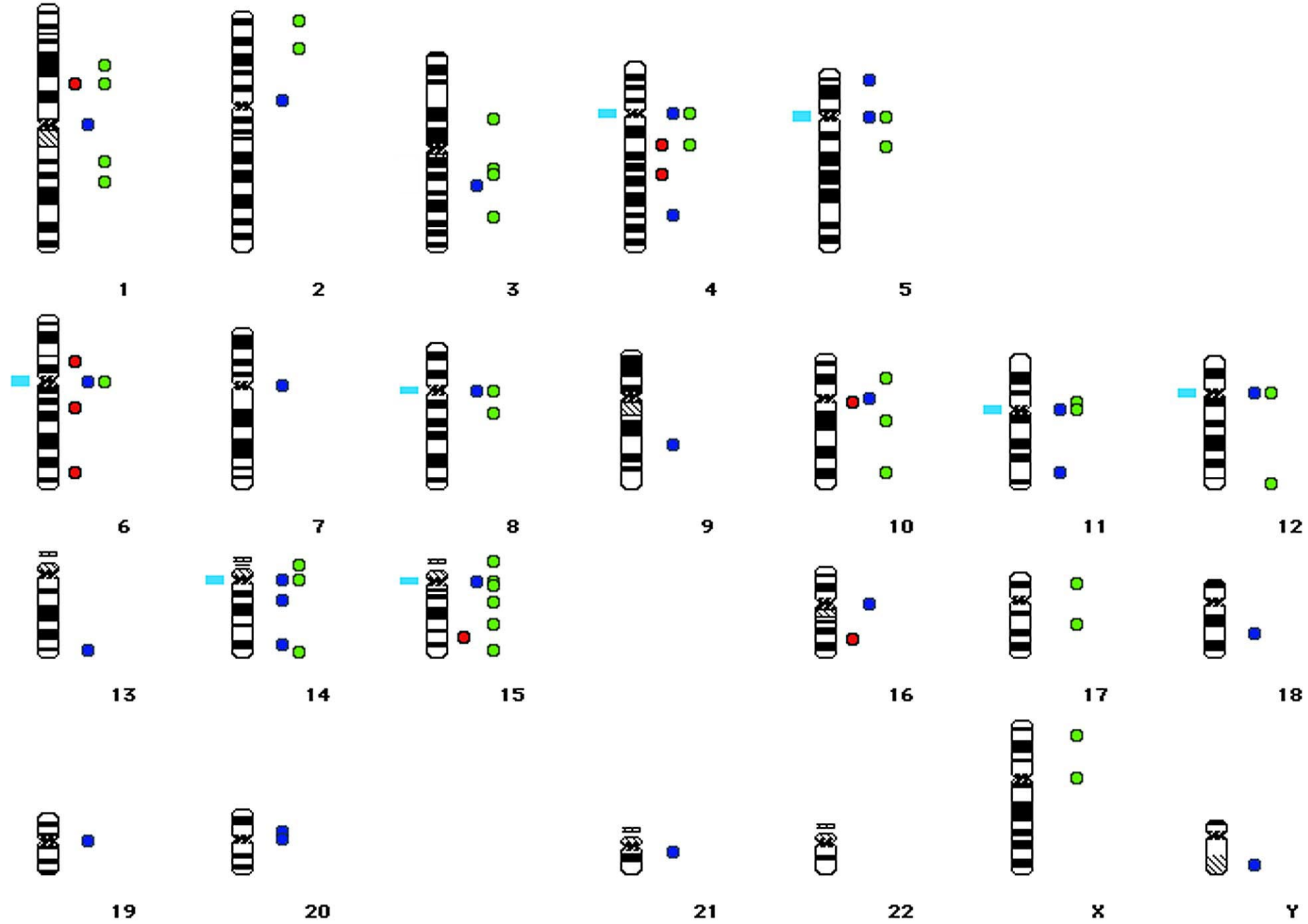

Figure 4 - Breakpoint analysis of the three CaP cell lines.