

Amplification of the Major Satellite DNA Family (FA-SAT) in a Cat Fibrosarcoma Might Be Related to Chromosomal Instability

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Abstract

Most mammalian chromosomes have satellite DNA sequences located at or near the centromeres, organized in arrays of variable size and higher order structure. The implications of these specific repetitive DNA sequences and their organization for centromere function are still quite cloudy. In contrast to most mammalian species, the domestic cat seems to have the major satellite DNA family (FA-SAT) localized primarily at the telomeres and secondarily at the centromeres of the chromosomes. In the present work, we analyzed chromosome preparations from a fibrosarcoma, in comparison with nontumor cells (epithelial tissue) from the same individual, by *in situ* hybridization of the FA-SAT cat satellite DNA family. This repetitive sequence was found to be amplified in the cat tumor chromosomes analyzed. The amplification of these satellite DNA sequences in the cat chromosomes with variable number and appearance (marker chromosomes) is discussed and might be related to mitotic instability, which could explain the exhibition of complex patterns of chromosome aberrations detected in the fibrosarcoma analyzed.

1. Introduction

Chromosomal instability is the gain and/or loss of whole chromosomes or chromosomal segments at a higher rate in a population of cells, such as cancer cells, compared to normal ones. Numerical and structural chromosomal alterations and chromosomal instability are common features of mammalian tumors. In most cases, aneuploidy is the result of numerical chromosomal alterations. Further segmental chromosomal gains and losses come from structural chromosomal alterations, including reciprocal and nonreciprocal translocations, homogeneously staining regions, amplifications, insertions, and deletions. Structural alterations may result in a further imbalance in gene expression, resulting in chromosomal instability (Gollin 2005). In some tumors, each cell within the tumor has a different karyotype due to chromosomal instability, which is thought to be the means by which cells develop the features that enable them to become cancer cells (Hanahan and Weinberg 2000).

Most mammalian chromosomes have satellite DNA sequences located at or near the centromeres, organized in arrays of variable size and higher order structure (Choo et al. 1991). These satellite sequences are widely thought to play a role in centromere activity (Tyler-Smith et al. 1998). Evidence that human alphoid repeats can induce novel centromere activity in chromosomes from other species (Larin et al. 1994) and provide centromeric function in artificial chromosomes (Harrington et al. 1997; Ikeno et al. 1998) indicate that a correlation exists between the presence of alphoid DNA and the formation of kinetochores. Other findings have shown this correlation to

be incomplete in dicentric chromosomes where centromeric activity is absent in one of the centromeres, implying a role for epigenetic factors in kinetochore formation (Choo 1997).

An association between amplified human alphoid DNA, generated by transfection of animal cells, and chromosomal instability was described by Haaf et al. (1992) and Heartlein et al. (1988). Gisselsson et al. (1999) described the behavior of human chromosomes containing amplified a-satellite sequences. These authors concluded that a propensity for additional kinetochore formation could be associated with the coamplification of alphoid DNA. Miki et al. (2004) reported for the first time, centrosome amplification as a cause of chromosomal instability in a feline lymphoma cell line.

In the genome of domestic cat, a major feline satellite DNA (FA-SAT) was described by Fanning (1987). These sequences seem to be present primarily at telomeres and secondarily at centromeres (Modi et al. 1988; Santos et al. 2004). Recently, Santos et al. (2004) presented an overall constitutive heterochromatin study with the physical mapping of the FA-SAT and telomeric sequences in cat chromosomes. The *in situ* hybridization of the cat satellite DNA and telomeric sequences, together with the general constitutive heterochromatin staining, allowed the FA-SAT family to be precisely localized in the cat chromosomes.

In the present work, we analyzed chromosome preparations from a cat fibrosarcoma, in comparison with nontumor cells (epithelial tissue) from the same individual, with *in situ* hybridization of the FA-SAT cat satellite DNA family and telomere sequences. This repeat sequence was found to

be amplified in the cat tumor cells. Here we discuss the amplification of these repeats in the cat fibrosarcoma chromosomes showing variable number and appearance. The amplification of the FA-SAT cat satellite DNA family might be related to mitotic and chromosomal instability and would explain the exhibition of complex patterns of chromosome aberrations detected in the fibrosarcoma analyzed.

2. Materials and Methods

Chromosome Preparations

The fibrosarcoma material obtained from a 7-year-old female cat and the normal epithelial tissue sample were surgically removed for cell culturing. The tumor tissue sample was processed for direct chromosome preparation and also for cell culturing (together with the normal tissue). After cell dissociation, the direct chromosome preparations were obtained with overnight colcemid treatment, followed by routine harvesting protocols. Explant cell cultures of the neoplasm and normal tissue were set up by mincing the solid tissues into small fragments. The fragments were transferred to sterile flasks containing 5 ml McCoy's medium (Invitrogen, Life Technologies, Groningen, the Netherlands) with L-glutamine, antibiotics (50 U penicillin/ml and 50 lg streptomycin/ml), and 10% fetal calf serum (all from Invitrogen, Life Technologies). The primary cultures were performed in 5% CO₂/air at 37 C for 10 days. Colcemid was added at a final concentration of 0.1 lg/ml for 5 h before harvesting.

GTD-banding

Air-dried slides were aged at 65 C for 5 h or overnight and then submitted to standard procedures of G-banding with trypsin. The chromosome preparations were fixed with paraformaldehyde as described before (Chaves et al. 2004) and subjected to sequential fluorescent in situ hybridization (FISH) procedures. Inversion of the 4#,6-diamidino-2-phenylindole (DAPI) color in Adobe Photoshop (version 5.0) revealed the chromosome's G-banding (GTD-banding, G-bands by trypsin with DAPI) for its identification. The organization of the karyotypes was done following the recommendations of Cho et al. (1997).

Probe Preparation and FISH

Isolation and characterization of the cat satellite DNA sequences are described in Santos et al. (2004). The analyzed clone proved to be a member of the FA-SAT family (sequence data available in the GenBank database with the accession number AY425348) and was used for FISH experiments.

Chromosome preparations were hybridized with the FA-SAT DNA sequences and telomeric probes after polymerase chain reaction labeling with biotin-16-dUTP (Sigma-Aldrich, Steinheim, Germany) or digoxigenin-11-dUTP (Roche, Molecular Biochemicals, Mannheim, Germany). In situ hybridization procedures were done using standard procedures. Biotin-labeled probes were

detected with avidin conjugated to fluorescein isothiocyanate (Vector Laboratories, Burlingame, CA) and digoxigenin labeled probes with 5-carboxy-tetramethylrhodamine conjugated to anti-digoxigenin (Roche, Molecular Biochemicals).

Chromosome Observation

Chromosomes were observed with a Zeiss Axioplan 2 Imaging microscope coupled to Axiocam digital camera and AxioVision software (version 2.0.5—Zeiss).

3. Results

A cat (*Felis catus*) fibrosarcoma was analyzed regarding chromosome FA-SAT in situ hybridization patterns and telomere sequences. As the tumor karyotypes from these fibrosarcoma cells were much rearranged, we decided to hybridize FA-SAT DNA sequences on the metaphases because this satellite DNA sequence produces an "almost" longitudinal differentiation for each cat chromosome, suitable for chromosome identification, as described by Santos et al. (2004).

In Figure 1, we show a normal karyotype (2n 5 38) (from normal epithelial tissue of the cat in analysis) with the in situ hybridization of the FA-SAT (Figure 1a) and telomeric (Figure 1b) probes. The in situ hybridization patterns detected in the normal tissue are essentially the same as the ones reported by Santos et al. (2004), namely, the FA-SAT family was apparently not detected in cat chromosomes A1, A2, B1, B4, C1, C2, and D3, and the other chromosomes showed similar localizations of the FA-SAT, as previously reported.

The analysis of at least 250 metaphases spreads from direct and culturing cells demonstrated the occurrence of several clones with marker chromosomes, which were identified only by their structure and satellite DNA longitudinal pattern. Regarding these identification items, the markers seem to be the same in each representative clone observed. The GTG-banding (G-banding by trypsin and Giemsa staining) demonstrated to be inadequate for the precise identification of these complex rearranged marker chromosomes.

Some of the most representative (with more than 50% of incidence) cell tumor clones detected (2n 5 49, 2n 5 50, 2n 5 32, and 2n 5 26) are shown, respectively, in Figure 2a,e, 2b,c,2d, and 2f. Figure 2a,b shows the G-banding of two representative metaphases of the cat tumor with marker chromosomes. An interesting quadriradial configuration can be observed in Figure 2a, characteristic of tumor cells with highly rearranged chromosomes. FA-SAT and/or telomeric probes in situ hybridization to the cells and chromosome preparations can also be observed in Figure 2, where a metaphase with FA-SAT hybridization (Figure 2c) and the corresponding GTD-banding (Figure 2b) are shown. In Figure 2b–f is evident (arrows) the presence of several marker chromosomes with aberrant FA-SAT in situ hybridization signals (cf. Figure 1 for standard patterns). The telomeric probe labeled the telomeres and also some interstitial regions (Figure 2d,f). Several chromatin strings

in interphase nuclei (Figure 2g) were also detected in the direct chromosome preparations. Finally, Figure 2h (G-banding) and the corresponding figure (Figure 2i), in situ hybridized with FA-SAT, highlight some of the marker chromosomes found in the most representative cell tumor clones detected.

4. Discussion

We analyzed the chromosomes of a feline fibrosarcoma with FA-SAT and telomeric probes. In Figure 1, it is possible to observe standard FISH distributions (according to Santos et al. 2004) to cat normal chromosomes for both sequences. In contrast, tumor chromosome preparations (from direct and culturing cells) revealed several marker chromosomes with aberrant FA-SAT in situ hybridization signals (Figure 2b–f,h,i; cf. Figure 1 for standard patterns). The marker chromosomes with clear satellite DNA sequences amplification represent an obvious evidence of chromosomal rearrangements and/or repetitive regions transposition events. This fact is also supported by the presence of telomere sequences at irregular interstitial regions in some of these chromosomes (Figure 2d,f). The amplification of satellite DNA sequences observed in chromosomes of variable number and appearance might be related with chromosomal instability and could explain the exhibition of complex patterns of chromosome aberrations. Furthermore, in the direct chromosome preparations, several chromatin strings in interphase nuclei were detected (Figure 2g), which reflect a high degree of mitotic instability (Gisselsson et al. 1999). To be clonally retained in the karyotype, the products of chromosomal rearrangements need at least one functional centromere and must be sufficiently capped by adequate telomeric repeats at their ends. Chromosomes with depleted telomeres have the propensity to be lost (Sandell and Zakian 1993), and more than one functional centromere initiates the breakage-fusion-bridge cyclic process that progressively generates ongoing chromosomal instability (Gagos and Irminger-Finger 2005). Our results demonstrate that in the tumor analyzed, there is evident chromosomal instability, revealed by the several marker chromosomes detected and the presence of chromatin strings between interphase nuclei. Gisselsson et al. (1999) analyzed similar events in a human atypical lipomatous tumor cell line with a-satellite sequences. These authors explained the chromosomal instability observed, as a propensity for additional kinetochore formation that could be associated with the coamplification of alphoid DNA and pericentromeric sequences. The a-satellite sequences analyzed by Gisselsson et al. (1999) are regularly found at the human centromeres. Our FA-SAT sequences are primarily found at the telomere regions and at the centromere region in some of the standard cat chromosomes (Modi et al. 1988; Santos et al. 2004). Nevertheless, in our experiments, the hybridization sensitivity would only detect a few kilobases of sequence; thus, a very small fraction of FA-SAT might be present at the chromosome centromeres (consisting of enough sequence elements for centromeric function). Furthermore, the FA-SAT sequences seem to be always

amplified at the centromere regions of the marker chromosomes, besides being or not being present at other chromosome regions. Hence, one can hypothesize that the large blocks of FA-SAT found, at least at the centromere region of the marker chromosomes, could be essential for centromere function, thus leading to the retention of these variable marker chromosomes. The presence of blocks of satellite DNA sequences in other regions of the marker chromosomes might be the result of chromosomal rearrangements. All these suppositions, at this stage of the work, are speculative. To attest them, the verification of additional kinetochore formation at these regions is needed. Also important would be the analysis of the marker chromosome's clonal evolution, besides the concomitant study of the amplified repetitive DNA sequences and centromeric activity elements, which could provide more insights into chromosomal instability and "tumorigenesis" process.

In summary, our data are demonstrative of FA-SAT sequence amplification associated with variable marker chromosomes, possibly related with chromosomal instability.

As far as we know, the phenomenon of satellite DNA sequences amplification in marker chromosomes, present in the direct and primary tumor cells, is here described for the first time in a *F. catus* tumor.

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References

- Chaves R, Santos S, and Guedes-Pinto H, 2004. Comparative analysis (Hippotragini versus Caprini, Bovidae) of X-chromosome's constitutive heterochromatin by in situ restriction endonuclease digestion: X-chromosome constitutive heterochromatin evolution. *Genetica* 121:315–325.
- Cho K-W, Youn H-Y, Watari T, Tsujimoto H, Hasegawa A, and Satoh H, 1997. A proposed nomenclature of the domestic cat karyotype. *Cytogenet Cell Genet* 79:71–78.
- Choo KH, 1997. Centromere DNA dynamics: latent centromeres and neo-centromere formation. *Am J Hum Genet* 61:1225–1233.
- Choo KH, Vissel B, Nagy A, Earle E, and Kalitsis P, 1991. A survey of the genomic distribution of alpha-satellite DNA on all human chromosomes. *Nucleic Acids Res* 19:1179–1182.
- Fanning T, 1987. Origin and evolution of a major feline satellite DNA. *J Mol Biol* 197:627–634.
- Gagos S and Irminger-Finger I, 2005. Chromosome instability in neoplasia: chaotic roots to continuous growth. *Int J Biochem Cell Biol* 37:1014–1033.

Gisselsson D, Höglund M, Mertens F, and Mandahl N, 1999. Variable stability of chromosomes containing amplified a-satellite sequences in human mesenchymal tumours. *Chromosoma* 108:271–277.

Gollin SM, 2005. Mechanisms leading to chromosome instability. *SeminCancer Biol* 15:33–42.

Haaf T, Warburton PE, and Willard HF, 1992. Integration of human a-satellite into simian chromosomes: centromere binding and disruption of normal chromosome segregation. *Cell* 70:681–696.

Hanahan D and Weinberg RA, 2000. The hallmarks of cancer. *Cell* 100:57–70. Harrington JJ, Bokkelen GV, Mayes RW, Gustashaw K, and Willard HF, 1997. Formation of the novo centromeres and construction of first-generation human artificial microchromosomes. *Nat Genet* 15:345–354.

Heartlein MW, Knoll JHM, and Latt SA, 1988. Chromosome instability associated with human alphoid DNA transfected into the Chinese hamster genome. *Mol Cell Biol* 8:3611–3618.

Ikeno M, Grimes B, Okasaki T, Nakano M, Saitoh K, Hoshino H, McGill NI, Cooke H, and Masumoto H, 1998. Construction of YAC-based mammalian artificial chromosomes. *Nat Biotechnol* 16:431–439.

Larin Z, Fricker MD, and Tyler-Smith C, 1994. De novo formation of several features of a centromere following introduction of a Y alphoid YAC into mammalian cells. *Hum Mol Genet* 3:689–695.

Miki R, Okuda M, Oikawa T, Watanabe M, Ma Z, Matsumoto K, Iwata H, and Inokuma H, 2004. Centrosome amplification and chromosomal instability in feline lymphoma cell lines. *J Vet Med Sci* 66(7):797–805.

Modi WS, Fanning TG, Wayne RK, and O'Brien SJ, 1988. Chromosomal localization of satellite DNA sequences among 22 species of felids and canids (Carnivora). *Cytogenet Cell Genet* 48:208–213.

Sandell LL and Zakian VA, 1993. Loss of a yeast telomere: arrest, recovery, and chromosome loss. *Cell* 75:729–739.

Santos S, Chaves R, and Guedes-Pinto H, 2004. Chromosomal localization of the major satellite DNA family (FA-SAT) in the domestic cat. *Cytogenet Genome Res* 107:119–122.

Tyler-Smith C, Cornish P, and Burns E, 1998. Neocentromeres, the Y chromosome and centromere evolution. *Chromosome Res* 6:65–71

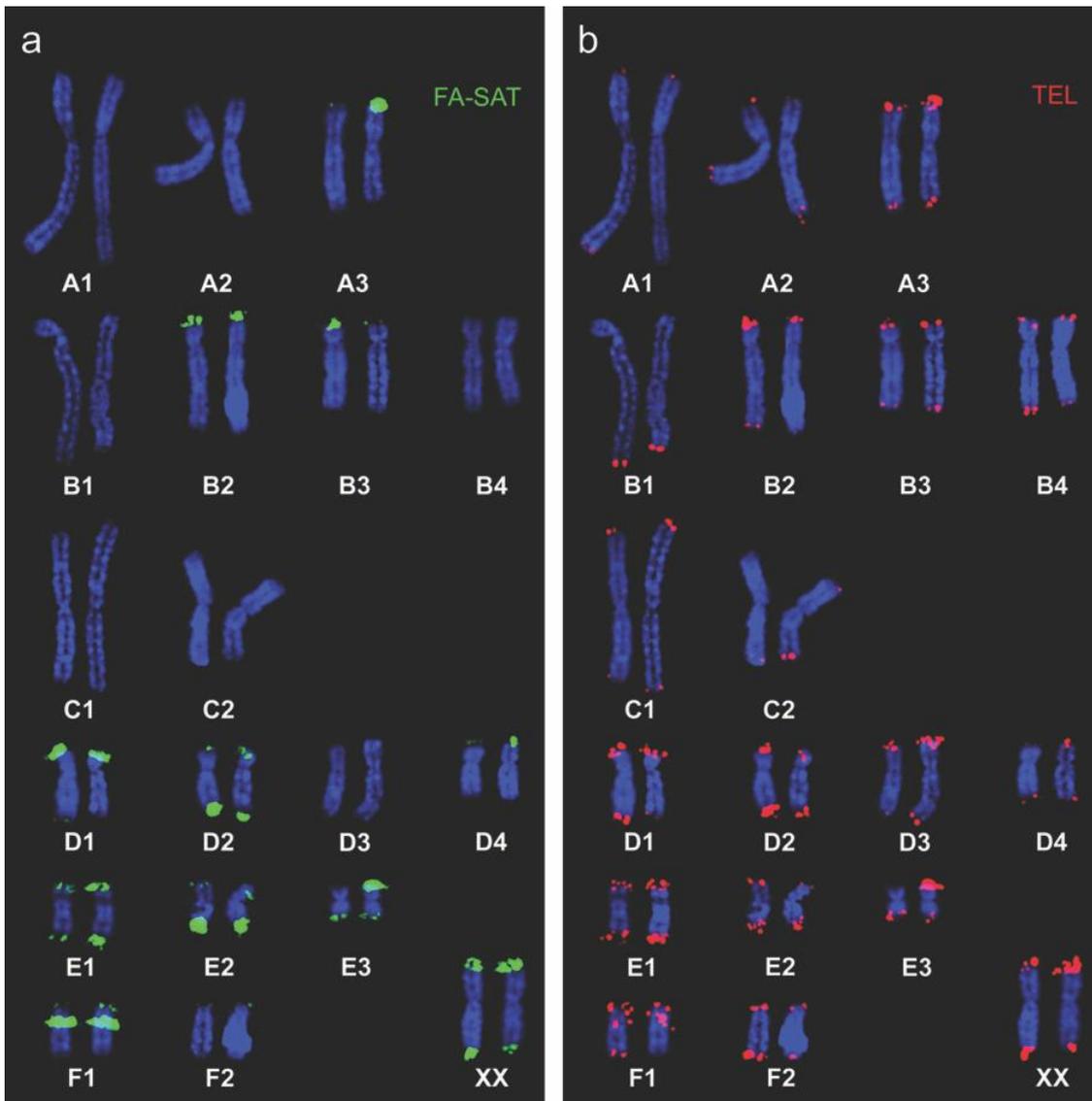


Figure 1. In situ hybridization of the FA-SAT (green signal, a) and/or telomeric probes (red signal, b) to chromosome preparations (counterstained in blue with DAPI) from normal tissue sample of the same cat with a fibrosarcoma. Normal cell's karyotypes were presented for an easier evaluation of the marker chromosomes identified with abnormal in situ hybridization patterns, detected in the tumor cells. Abbreviations: FA-SAT, major feline satellite DNA; TEL, telomeric probe.

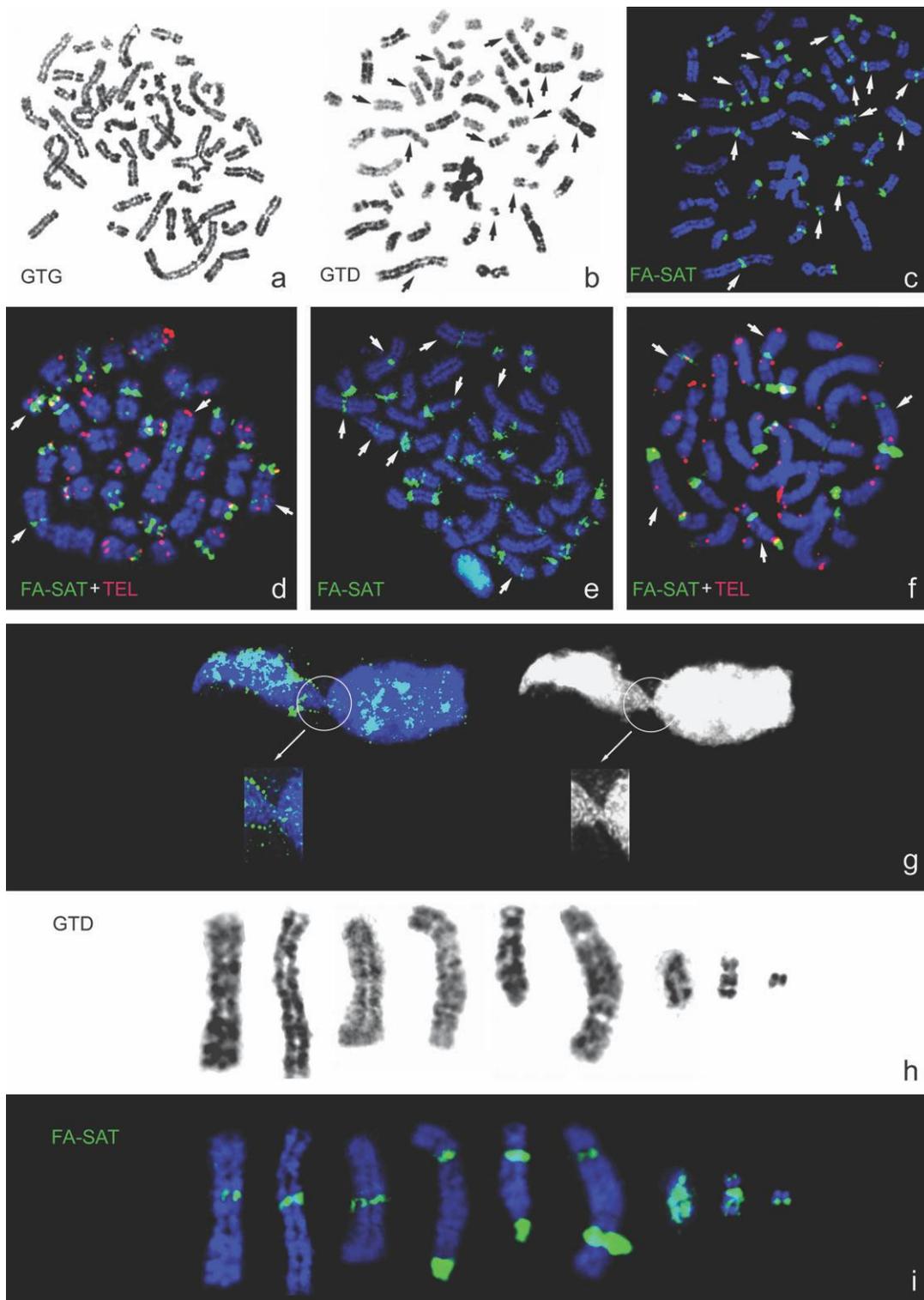


Figure 2. G-banding and in situ hybridization of the FA-SAT (green signal) and/or telomeric probes (red signal) to chromosome preparations and interphase nuclei (counterstained in blue with DAPI) from a cat fibrosarcoma. (a–e) Direct chromosome preparations. (f) Metaphase preparation obtained from cell culture. (g) Chromatin strings between interphase nuclei. (h,i) Marker chromosomes consistently detected in the metaphases analyzed. The small arrows show marker chromosomes identified in the metaphases in analysis (cf. Figure 1 for standard FA-SAT satellite DNA sequences patterns). Abbreviations: GTG, G-banding by trypsin and Giemsa staining; GTD, G-banding by trypsin and DAPI counterstaining; FA-SAT, major feline satellite DNA; TEL, telomeric probe.