Novel Putative Fragile Sites Observed in Feline Fibroblasts Treated with Aphidicolin and Fluorodeoxyuridine

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ABSTRACT. Fragile sites are non-randomly distributed chromosomal breaks and gaps observed in the cells cultivated under certain conditions. Feline fragile sites were analyzed using skin fibroblast strains after the treatments with aphidicolin and fluorodeoxyuridine in combination with caffeine. Three aphidicolin-induced fragile sites (A1q21, C2q13 and E1p21) as well as a folate-sensitive site (B1q14) were observed in all the 3 fibroblast strains tested for each treatment group. The loci in A1q21 and B1q14 are very close to that reported previously for peripheral blood lymphocytes and lung cells. Two chromosomal break points in C2q13 and E1p21 seem to be new fragile sites. Fifteen candidates for feline fragile sites were also assigned their locations in feline chromosomes. Both the incidence and distribution of feline fragile sites in skin fibroblasts seem to be different at least in part from those in lymphocytes. — KEY WORDS: aphidicolin, chromosomal break, feline, fluorodeoxyuridine, fragile site.

Fragile sites are non-random chromosomal breaks and gaps observed in the cells under the folate-deficient condition [25] as well as those treated with particular mutagen, carcinogen and clastogens [31]. A great number of papers has been reported the fragile sites in human chromosomes, and 116 sites have already been authorized and assigned their location to specific chromosomal bands until 1993 [24]. For domestic animals, however, only limited numbers of information are available [17–21, 23, 28]. So far, only two papers concerning feline fragile sites have been published. Stone et al. are the first to report 3 feline fragile sites commonly observed in blood lymphocytes of 3 domestic cats [21]. Using lung cell culture from a new born kitten, Ronne has detected 22 putative fragile sites[20]. Fragile sites have been milestones in the studies on the human diseases with chromosomal instability [25]. Though the significance of fragile sites in the development of cancer is still to be examined, the evidences of chromosomal instability in normal cells derived from cancer patients have been accumulated [2, 8, 14, 27]. It is important to assign the chromosomal break point frequency observed in a particular type of tumor in order to identify a specific gene which is responsible for the onset of the disease. Recently, the close correlations between specified fragile sites and particular neoplasms have been reported [9, 13, 26]. It is possible that more accurate information will reveal the direct correlation between fragile sites and the break points in chromosomes. These information would be also very helpful in the diagnosis and treatment of malignant tumors in domestic animals, especially in companion animals. Therefore, the determination of the precise locations of fragile sites are an urgent proposal in order to estimate their significance in the tumorigenesis in domestic animals.

Fragile sites in human chromosomes were studied most in peripheral blood lymphocytes. For the study on feline fragile sites, however, lymphocytes are less suited, because the culture often suffer a heavy loss of lectin-activated cells by aggregation with platelets. In human, the fragile sites in skin fibroblasts treated with aphidicolin differ in both frequency and distribution as compared with those observed in lymphocytes [10, 11]. In the present study, we have investigated novel fragile sites induced in feline skin fibroblasts, because the banding pattern of their chromosomes have been well-characterized [12].

MATERIALS AND METHODS

Feline fibroblast strains: Small patches of skin were excised from foot pads of four healthy female domestic cats under the sterile condition. The skin fragments were minced with scissors in the Ca²⁺- and Mg²⁺-free Dulbecco’s phosphate buffered saline (PBS(-)), and cell suspension obtained was inoculated into 25 cm² plastic culture flasks containing Eagle’s minimum essential medium (Nissui) supplemented with MEM sodium pyruvate solution (GIBCO), nonessential amino acid solution (GIBCO), and 10% inactivated fetal bovine serum (Hyclone). The flasks were incubated in the humidified atmosphere containing 5% CO₂ at 37°C and medium was replenished periodically until the growth of the fibroblasts reached confluence.

Drug treatment: Single-cell suspension was prepared from a confluent culture of fibroblasts by trypsinization and inoculated into a plastic dish containing 10 ml of the growth medium. The dish was then incubated under the condition mentioned above for 2 days. Drug treatment was carried out by the procedure described by Yunis and Soreng with slight modifications [30]. Briefly, the log-phase culture of fibroblast strains FFB2, FFB3 and FFB4 were treated with...
25 ng/ml fluorodeoxyuridine (FUdR) for 18 hr. Caffeine was, then, added to the culture at 2.2 mM and the treatment continued for additional 6 hr. Alternatively, the exponentially growing cells from strains FFB1, FFB3 and FFB4 was treated with 50 ng/ml aphidicolin (Wako Pure Chemical Co.) for 24 hr. For chromosome analysis, colcemid was added at 0.05 mg/ml 30 min prior to the termination of the drug treatments.

Analysis of break points in feline chromosomes: The drug-treated cells were harvested by trypsinization and resuspended in hypotonic solution (0.075 M KCl). After the hypotonic treatment for 10 min at room temperature, the cells were fixed by addition of ice-cold acetic acid-methanol (1:3) solution. The fixation was completed by changing the fixative for several times. Cell suspension in the fixative were dropped onto slides and air-dried [6, 7]. The slides thus prepared were stained with quinacrine mustard-Hoechst 33258 solution [29].

The chromosome preparation was examined using a fluoromicroscope (Nikon Optiphoto). Well-spread metaphases were photographed and analyzed after Q-band karyotyping. Chromosomal breaks and gaps were identified their localization in the chromosomes [3, 12]. Mostly, more than 50 metaphase cells were analyzed for each strains.

RESULTS

Figure 1 shows a typical Q-banded metaphase cell treated with aphidicolin. Thirty three intact chromosomes and 5 chromosomes containing isochromatid breaks/gaps were observed. Analysis of karyotypes revealed that the breaks/gaps are present in chromosomes A1p, C2q (both C2 chromosomes are affected), E1p and E3p.

Figure 2 summarizes the locations of breaks or gaps observed in chromosomes of the 260 feline fibroblasts examined. The result includes all the 86 break points induced by both aphidicolin and FUdR-cafeine. Of these locations, no. 1, no. 8 and no. 27 correspond with the fragile sites observed by Rønne [20]. So far, loci in bands C2q13 and E1p21 should be counted as new fragile sites in fibroblasts.

Additional candidates of feline fragile sites are listed in Table 3. Thirteen sites were observed at the frequency higher than 4% in at least one fibroblast strain. Of these sites, loci no. 3 and no. 69 showed high incidence of aphidicolin-induced gaps and breaks in FFB1 and FFB4.

DISCUSSION

Large number of fragile sites have been reported for human chromosomes [24], while the studies on those in domestic animals are limited [17–21, 23, 28]. For domestic cat, only 2 reports have been published. Stone et al. have firstly reported 3 fragile sites commonly observed in feline lymphocytes from 3 healthy donnors [21]. Rønne has identified 22 fragile sites in methotrexate/bromodeoxyuridine-treated lung cells from a normal newborn kitten [20].

In the present study, we detected 3 aphidicolin-induced putative fragile sites in all the 3 feline fibroblast strains examined. At least 2 of these are different from those identified previously [20, 21]. It is important to determine the locations of fragile sites in the cells from different tissues to rationalize the chromosomal break points in tumors of domestic animal. As suggested by Murano et al. for human cells [10, 11], the distribution and frequency of aphidicolin-induced fragile sites also seems to be different between lymphocytes and fibroblasts of domestic cats. Stone et al. have shown that there is canine breed specific variation in the frequency of aphidicolin-inducible fragile sites [22]. Because a similar variation in domestic cats with different genetic background is possible, more detailed studies on the feline chromosomal stability is required for understanding of feline fragile sites.

All the fibroblast strains used in this study were established from unrelated domestic cats. Because all the human fragile sites are not always expressed in all individuals, the loci listed in Table 3 may also be a potential
candidates for feline fragile sites. Interestingly, 7 sites of these were observed at relatively high frequency by Rønne [20]. It is widely accepted the incidence of folate-sensitive fragile sites is greatly dependent on the intracellular pool size of both folate and nucleotides[4, 5, 16]. Because the variation of these quantities among a variety of tissues is highly probable, the incidence of fragile sites may vary significantly among the different types of cells employed.

At present, only 26 feline fragile sites have been identified including 2 novel sites observed. Though the information on the fragile sites in domestic cats are very limited, it is probable that the low incidence of feline fragile sites, as
compared with that in human, may have resulted from the selective elimination of inferiors in establishing the breeds. Alternatively, it is possible that the cellular sensitivity to fluorodeoxyuridine plus caffeine and aphidicolin may differ among mammalian species. Conventionally, same procedure and drug concentrations as those for the detection of human fragile sites have been employed to study those in domestic animals. Precise dose-induction relationship of fragile sites for each mammalian species, therefore, should be established.

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REFERENCES


