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HIGH RESOLUTION KARYOTYPE OF THAI CRAB-EATING MACAQUE (*MACACA FASCICULARIS*)

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Abstract - Comparative chromosome banding analysis and/or fluorescence in situ hybridization (FISH) studies are established approaches to compare human and ape chromosomes. FISH banding is a relatively new and not routinely applied method very well suited to provide to a better understanding of the evolutionary history of primate and human phylogeny. Here multicolor banding (MCB)-applying probes derived from *Homo sapiens* were used to analyze the chromosomes of Thai crab-eating macaque (*Macaca fascicularis*). The results agree with those of previous studies in other macaques, e.g. *Macaca sylvanus* or *Macaca nemestrina*. This result highlights that morphological differences within the Cercopithecoidea must be found rather in subchromosomal changes or even in epigenetics than in gross structural alterations.

Key words: Macaca fascicularis; karyotype; multicolor banding; fluorescence in situ hybridization (FISH)

INTRODUCTION

Since recently next-generation sequencing (NGS) was introduced to answer the question what the genetic differences between species are, molecular cytogenetic studies to contribute to a better understand of the evolutionary history of primate and human phylogeny seem to be outdated. However, basic cytogenetic data are needed for the exact alignment of these new complex datasets, as outlined by Zhang et al. (2012).

Pure banding cytogenetic studies are still the starting point in many species (e.g. Supiwong et al., 2013). In primates, similar studies were performed in the 1980s and 1990s (Yunis and Prakash, 1982; Morescalchi et al., 1998). After the introduction of multicolor-fluorescence in situ hybridization (FISH), FISH studies using whole chromosome painting probes and FISH-banding approaches (Liehr et al., 2006) have been done successfully. However, FISH-banding approaches were not used systematically to study the question of karyotype evolution in primates; only occasionally a few studies were performed (de Oliveira et al., 2002; Müller and Wienberg, 2001, Müller et al., 1998). The most frequently applied FISH-banding approach is the so-called multicolor banding (MCB), which has the unique feature of be-

ing anchored in the human DNA-sequence (Weise et al., 2008). MCB has already been used for the comparative molecular cytogenetic mapping of the following primate species: *Gorilla gorilla* (Mrasek et al., 2001), *Hylobates lar* (Mrasek et al., 2003), *Trachypithecus cristatus* (Fan et al., 2013), *Macaca sylvanus* (Fan et al., in press 1), and *Macaca nemestrina* (Fan et al., in press 2).

Here the first MCB-based study for the characterization the karyotype of crab-eating macaque *Macaca fascicularis* (MFA) is presented. During the Pliocene or Pleistocene, i.e. during the last 3-5 million years, macaques (Catarrhini; Cercopithecoidea; Cercopithecinae; Papionini) underwent rapid radiation in Africa and especially Asia (Camperio Ciani et al., 1989). Macaques are a morphologically highly diverse group. According to the literature, on the chromosomal level this group remained unchanged: 42 chromosomes with no differences at the cytogenetic level (Brown et al., 1986). Thus, in captivity different macaque species can form hybrids, even fertile ones (Moore et al., 1999).

MFA was previously studied by banding cytogenetics (Fernandez-Donoso et al., 1970, Kanagawa et al., 1971; Brown et al., 1986); to the best of our knowledge, FISH was only applied for single loci (Archidiacono et al., 1998; Kasai et al., 2000; Kostova et al., 2002; Ruiz-Herrera et al., 2004; Liu et al., 2007) and not for the whole genome. In addition, a new alphoid DNA sequence was isolated from MFA and used in FISH (Crovella et al., 1999). Here we provide the first genome-wide MCB-based FISH-banding study in MFA.

MATERIALS AND METHODS

Five milliliter of heparinized peripheral blood of one male MFA was acquired in Thailand. Blood lymphocytes were subjected to short-term culture and cytogenetic work-up under standard conditions.

Twenty-four multicolor banding (MCB) probes derived from human *Homo sapiens* (HSA) chromosomes were applied in 24 independent FISH experiments in MFA chromosome preparations as previously reported (Mrasek et al., 2001). Evolutionary conserved chromosomal breakpoints were characterized with respect to the human chromosome complement (Table 1).

RESULTS AND DISCUSSION

In MFA, 43 evolutionary conserved breaking events were identified using human karyotype as an equation (Table 1). It should be noted that the nomenclature of macaque chromosomes used here was adapted from Morescalchi et al. (1998), as there are different nomenclatures in circulation, e.g. MFA chromosomes 12 and 13 are designated as 9 and 15, respectively (Ruiz-Herrera et al., 2002). The evolutionary conserved breaking events in MFA (Table 1) were identical to those known from other macaque species living in parts of western Africa (Ventura et al., 2007; Fan et al., in press 1). They remained stable even though MFA (this study) and *Macaca nemestrina* (Fan et al., in press 2) are living in Thailand, i.e. thousands of kilometers apart.

In addition, MCB using human probes left unstained some regions in MFA. Those were most likely species-specific amplifications of unknown genetic material in regions homologous to HSA 9q34, 17p10 and 17q24. For 17q24 complex regions of segmental duplication were previously reported (Cardone et al., 2008). On the other hand, HSA-specific amplicons present in 1q12, 9q12, 16q11.2 and Yq12 were not present in MFA. These observations go together well with the idea that species-specific amplifications might play a major role in speciation (Mrasek et al., 2001). Further studies of the macaque specific amplicons might mead to interesting results in future.

Finally, the centromeric regions in MFA 3, 5, 6, 8-11, 16, 19, 20, X and Y were identical to human centromeric positions (Table 1). However, the centromeric regions, even if intraspecifically stable, do not contain sequence-identical alphoid DNA stretches. These regions are fascinating from the evolutionary point of view, as it is suggested that these chromosomal regions evolve faster than other genomic

Table 1. Evolutionary conserved breaks in MFA according to MCB

MFA	cen	MFA chromosomes given as derivatives of human chromosomes
1	1q42.13	inv(1)(q23.3q42.13),dim(1)(q12)
2	3q26.1	der(3)(qter->q27.3::p22.3->p24::q22.1->q27.3::p22.3->p12.3::p26.3->p24::q22.1->p12.3:)
3	like HSA 7	der(7)(21qter->21q11.2::7p22.3->7p22.1::7q21.3->7q22.1::7q11.23->7p21.3::7p21.3->7q11.23::7q22.1->7qter)
4	6q24.3	inv(6)(p24q25.2) and inv(6)(q21q25.2)
5	like HSA 4	inv(4)(p15.3q10)
6	like HSA 5	no change to HSA 5
7	15q25	der(15)t(14;15)(q11.2;q26.3)
8	like HSA 8	no change to HSA 8
9	like HSA 10	inv(10)(q11.23q22.3)
10	like HSA 22	der(20)(22qter->22p13::20p11.21->20p13::20q11.21->20qter)
11	like HSA 12	no change to HSA 12
12	2q22.1	inv(2)(q14.1q21.1)
13	2p11.2	inv(2)(q11.1q14.1)
14	11p15.4	inv(11)(p15.4q13.4)
15	9q33.2	der(9)(9qter->9q34::?::9q34->9p24.3::9q21.11->9q22.33:),dim(9)(q12)
16	like HSA 17	der(17)(pter->p10::?::p10->q12::q23.3->q21.32::q12->q21.32::q23.3->q24::?::q24->qter)
17	13q21.31	no change to HSA 13
18	18q21.2	no change to HSA 18
19	like HSA 19	no change to HSA 19
20	like HSA 16	inv(16)(q22.1q22.3),dim(16)(q11.2)
X	like HSA X	no change to HSA X
Y	like HSA Y	del(Y)(q12q12)

cen – centromeric position; HSA – *Homo sapiens*

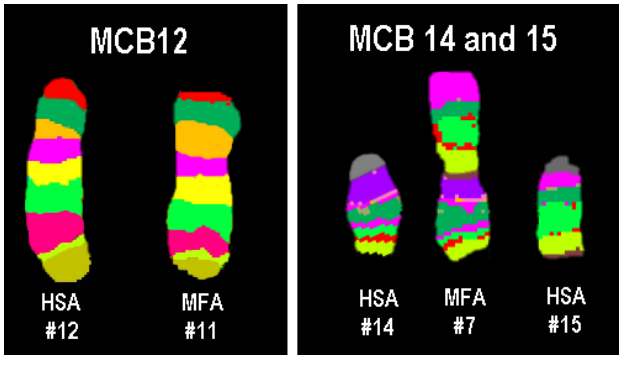

Fig. 1. Representative examples for MCB-result obtained in MFA. A - MCB 12 probe set applied on a human chromosome 12 (HSA) compared to the result obtained on homologous MFA chromosome 12. No differences were observed. B - MCB 14 and 15 applied on corresponding human chromosomes and on homologous MFA chromosome 7: a fusion of both chromosomes is observable. The acrocentric short arms of both chromosomes present in HSA are absent in MFA.

regions (Archidiacono et al., 1995). For centromeric positions of MFA 1, 2, 4, 7, 12-15, 17 and 18, so-called centromere repositioning was observed (Table 1), as discussed elsewhere (Rocchi et al., 2012).

CONCLUSION

Based on MCB, in MFA an identical karyotype was determined as in our previous studies of *Macaca sylvanus* and *Macaca nemestrina*. Thus, at the (molecular) cytogenetic level, no differences between these three macaques were found, even though differences in macaque-specific amplicons cannot yet be excluded. Nonetheless, our studies confirmed that the general chromosomal composition cannot be the underlying biological basis for the radiation and specification in Cercopithecoidea.

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