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Original scientific paper

HIGH RESOLUTION KARYOTYPE OF THAI CRAB-EATING MACAQUE (*Macaca fascicularis*)

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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 suited very well 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* was 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 pinpoints, that morphological differences within the Ceropithecoidae must be founded rather in subchromosomal changes or even in epigenetics than in gross structural alterations.

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

INTRODUCTION

As recently next generation sequencing was introduced to answer the question what are the genetic differences between the species, molecular cytogenetic studies to contribute to a better

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understand of the evolutionary history of primate and human phylogeny seem to be outdated. However, basic cytogenetic data is needed essentially for exact alignment of these new complex datasets as outlined by ZHANG *et al.* (2012).

Pure banding cytogenetic studies still are the starting point in many species nowadays (e.g. SUPIWONG *et al*., 2013). In primates similar studies were performed in the 1980s and 1990s (MORESCALCHI *et al.,* 1998). After introduction of multicolor-fluorescence in situ hybridization (FISH), FISH studies using whole chromosome painting probes and FISH-banding approaches (LIEHR *et al*., 2006) were done successfully. However, the FISH-banding approaches were not used systematically to study the question of karyotype evolution in primates; only occasionally a few studies were done (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 being anchored in the human DNA-sequence (WEISE *et al*., 2008). MCB was already used for comparative molecular cytogenetic mapping of the following primate species before: *Gorilla gorilla* (MRASEK *et al*., 2001), *Hylobates lar* (MRASEK *et al.,* 2003), *Trachypithecus cristatus* (FAN *et al*., 2013), *Macaca sylvanus* (FAN et al., 2014a), and *Macaca nemestrina* (FAN *et al.,* 2014b).

Here the first MCB-based study for the characterization the karyotype of crab-eating macaque (*Macaca fascicularis* = MFA) is presented. During Pliocene or Pleistocene, i.e. during the last 3-5 million years Macaques (Catarrhini; Ceropithecoidae; Cercopithecinae; Papionini) underwent a quick radiation in Africa and especially Asia (FAN *et al*., 2014a and b). Macaques are a morphologically highly diverse group. According to the literature, on the chromosomal level this group kept absolutely constant: 42 chromosomes with no differences on the cytogenetic level. Thus, in captivity different macaque species can form hybrids, even fertile ones (MOORE *et al.*, 1999).

MFA was studied before by banding cytogenetics (FERNANDEZ-DONOSO *et al.,* 1970, KANAGAWA *et al.,* 1971); to the best of our knowledge FISH was only applied for single loci (KASAI *et al*., 2000; KOSTOVA *et al*., 2002; RUIZ-HERRERA *et al*., 2004; LIU *et al.,* 2007) and not for the whole genome yet, also 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 heparinzed 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.

24 multicolor banding (MCB) probes derived from human chromosomes *Homo sapiens* (HSA) 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 (see Tab. 1).

RESULTS AND DISCUSSION

In MFA 43 evolutionary conserved break-events were identified sing human karyotype as an equation (Tab. 1). It has to be annotated that the nomenclature of macaque chromosomes used here was adapted from MORESCALCHI *et al*. (1998), as there are different nomenclatures around;

e.g. MFA chromosomes 12 and 13 are designated as 9 and 15, respectively, elsewhere (RUIZ-HERRERA *et al*., 2002). The evolutionary conserved break-events in MFA (Tab. 1) were identical to those known from other macaque species, living in parts in Western Africa (VENTURA *et al*., 2007; FAN *et al.*, 2014a). They remained stable even though MFA (this study) and *Macaca nemestrina* (FAN *et al.*, 2014b) are living in Thailand, i.e. thousands of kilometers apart.

Table 1. Evolutionary conserved breaks in MFA acc. 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)

Besides, 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 reported previously (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 are 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 (Tab. 1). However, the centromeric regions, even if being intraspecifically stable do not contain sequence identical alphoid DNA stretches. Also these regions are fascinating from the evolutionary point of view, as it is suggested that these chromosomal region evolve faster than other genomic regions. For centromeric positions of MFA 1, 2, 4, 7, 12-15, 17 and 18 so-called centromere repositioning was observed (Tab. 1), as discussed elsewhere (ROCCHI *et al.,* 2012).

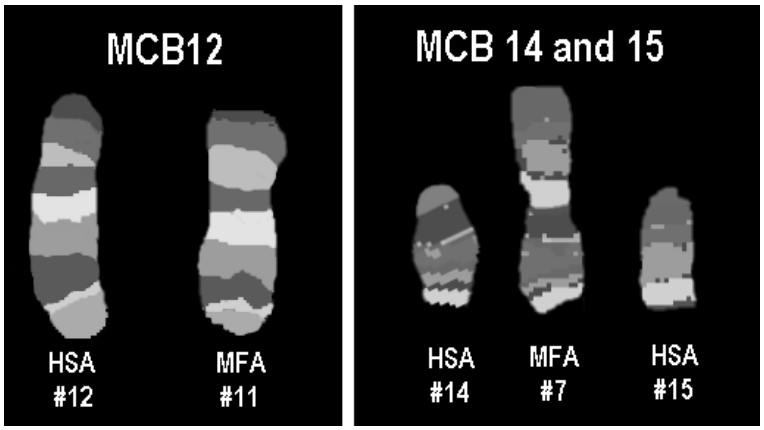

Figure 1.Representative examples for MCB-result obtained in MFA.

A) Result of 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.

CONCLUSION

Based on MCB in MFA the identical karyotype was determined as in our previous studies in *Macaca sylvanus* (FAN et al., 2014a) and *Macaca nemestrina* (FAN et al., 2014b). Thus, at (molecular) cytogenetic level no differences between these three macaques were found, even though differences in macaque-specific amplicons cannot be excluded yet. Still, our studies confirmed that the general chromosomal composition cannot be the underlying biological basis for the radiation and specification in Ceropithecoidae.

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KARIOTIP VISOKE REZOLUCIJE MAKAK RAKOJEDA (*Macaca fascicularis*)

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Izvod

komparativna hromozomska analiza i/ili in situ fluoroscentna hibridizacija (FISH) se koriste za poređenje ljudske i hromozome majmuna. FISH-tehnologija je relativno nova I ne koristi se rutinski is a relatively new and not routinely applied method koja može da obezbedi veću razumevanje evolucije primata i ljudsku filogeniju. Višebojne trakaste probe derivati iz *Homo sapiens* su korišćeni za analizu Thai makak rakojeda (*Macaca fascicularis*). dobijeni rezultati su u saglasnosti sa ranijim ispitivanjima drugih makaki, *Macaca sylvanus* ili *Macaca nemestrina*. ovi rezultati ukazuju da morfološke razlike unutar Ceropithecoidaemorjau biti zasnovane pre na subhromozomskim promenamili čak epigenetici nego u velikim strukturalnim promenama.

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