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Article

Genomic Tools for the Use of Nonhuman Primates in Translational Research

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Abstract

Nonhuman primates (NHPs) are important preclinical models for understanding the etiology of human diseases and for developing therapies and vaccines to cure or eliminate disease. Most human diseases have genetic components. Therefore, to be of maximal utility, the NHP species used for translational science should be as well characterized in regard to their genome and transcriptome as possible. This article reviews the current status of genomic information for the five NHP species used most often in translational research: rhesus macaque, cynomolgus macaque, vervet (African green) monkey, baboon, and marmoset NHP. These species have published whole genome sequences (with the exception of the baboon) and relatively well-characterized transcriptomes. Some have also been characterized in regard to specific genetic loci that are particularly related to translational concerns, such as the major histocompatability complex and the cytochrome P40 genes. Genomic resources to aid in stratifying captive populations in regard to genetic and phenotypic characteristics have been developed as an aid to enhancing reproducibility and facilitating more efficient use of animals. Taken together, the current genomic resources and numerous studies currently underway to improve them should enhance the value of NHPs as preclinical models of human disease.

Key words: nonhuman primate; genome; transcriptome; rhesus; cynomolgus; vervet; baboon; marmoset

Introduction

Nonhuman primates (NHPs), and monkeys in particular, are important preclinical models for understanding the etiology of human diseases and for developing therapies and vaccines to cure or eliminate disease (Capitanio and Emborg 2008; Izpisua Belmonte et al. 2015; Phillips et al. 2014). A white paper released by the Foundation for Biomedical Research in August 2016, sponsored by eight scientific societies, notes many scientific breakthroughs that have required the use of NHPs as preclinical models, dating from the 1950s to the present (National Association for Biomedical Research 2016). Areas of translation requiring NHPs span all fields of modern medicine, for example: infectious disease, including HIV/AIDS and emerging pathogens such as Ebola and Zika viruses; neurobiology, including neurodegenerative diseases such as Parkinson's and Huntington's disease; vision, including macular degeneration and treatment of congenital cataracts; metabolism, including diabetes; cardiovascular disease, including hypertension and atherosclerosis; and reproduction, including polycystic ovary syndrome and endometriosis. A list of all subject areas and diseases studied using NHPs would be very long.

It is not the intent of this review to discuss the details of NHP-based translational studies nor to justify the use of NHPs for translational research, as is clear from the many advances related to understanding aspects of human health and ameliorating disease that have been facilitated by the use of these animals as preclinical models. Rather, this review will provide an overview of the genomic and genetic resources available to the research community to facilitate the use of the five major species of NHPs that are currently used as preclinical models.

A previous volume of the *ILAR Journal* (volume 54, number 2, November 2013) was devoted to the overall topic of NHP genetics and genomics and contains considerable detail, not possible to cite in a single article, on this subject.

This review will discuss only monkeys as preclinical models. Chimpanzees were critical in the past for certain preclinical studies, for example, the development of hepatitis vaccines (Purcell and Emerson 2011). Research use of chimpanzees owned or supported by the US National Institutes of Health (NIH) has been prohibited, thereby removing them from biomedical research. Therefore, information on the genomics of the great apes will not be discussed here, as it now has very limited relevance to translational studies.

Most, if not all, human diseases have genetic components, the effects of which are influenced by environment. The sequencing of the human genome as well as development of tools such as the HapMap (International HapMap Consortium 2007) were motivated to help understand the genetic basis of human disease and to facilitate development of therapies. Likewise, it is a reasonable assumption that an understanding of the genetic determinants that can influence the physiology of the NHPs that are used as preclinical models will be of major utility in translational investigations using these animals. This consideration has been one of the major motivations for sequencing the genomes of NHPs such as the rhesus and cynomolgus macaques, as discussed in more detail, below. It should be noted, however, that the utility of an animal model does not necessarily depend on having a detailed knowledge of its genetic constitution. For example, the polio vaccines tested in large numbers of rhesus monkeys as preclinical models (Sabin 1985; Salk and Salk 1984) and the hepatitis vaccines tested in chimpanzees (Purcell and Emerson 2001) were developed largely without reference to the genetics of the preclinical animal models.

Considered broadly, characterization of the genetics of NHPs, including genome sequences, can influence translational research in the following ways. (1) Comparison of the genome sequences of humans and NHPs can provide insights into whether, for specific genomic regions, there is sufficient homology to justify the use of the NHP as a model of the human. Also central to this approach is to attain as complete knowledge as possible regarding the sequence variation within the NHP species being used to understand the potential effects of genetic heterogeneity on the specific disease being studied. Comparative studies can also be extended to include analysis of patterns of gene expression in the normal and diseased states as a means of using the NHP to inform investigations in the human. (2) NHP models of specific diseases can be developed either by finding and breeding natural variants within captive populations or by genetic modification. Findings in the NHP model can be hypothesis-generating for better understanding the disease in the human. (3) Like humans, NHPs are outbred populations. Therefore, there may be aspects of the genetic background of the NHP that can influence the expression of a specific gene or pathway that is potentially disease related in the human or related to immune responses to pathogens or vaccines.

Issues around the necessity of using large populations of well-characterized captive-bred NHPs in preclinical research are exacerbated by the cost of experiments and the availability of animals. Experiments must usually be performed with limited numbers of animals. Therefore, at the very least, known genes that can affect an experimental result must be characterized within the experimental cohort of animals used. An example of this is the confounding effect that specific alleles of the major histocompatibility complex (MHC) can have on HIV/AIDS vaccine trials using monkeys (Loffredo et al, 2007; Mothé et al, 2002). Therefore, genetic analysis of the captive monkey populations used for experiments can help reduce experimental variation and enhance reproducibility.

In addition to the genetic resources discussed below, other factors facilitate the use of NHPs as preclinical models. First, the environmental conditions, including diet, in which NHP colonies are housed and bred provide a standardized milieu, which can ameliorate some of the variables inherent in human clinical populations. Second, extensive physical and intellectual resources are available to facilitate translational research using NHPs. Husbandry and breeding of NHPs is expensive and specialized. Therefore, centralized resources that house and characterize NHP subjects for preclinical investigations and are available for use by a variety of preclinical researchers are essential. Examples of resources in the US are the seven National Primate Research Centers (NPRCs), the New Iberia Research Center, the Keeling Center for Comparative Medicine and Research, the Caribbean Primate Research Center, and the Wake Forest University Primate Center. In addition to these academic centers, several contract research organizations in the United States house, and in some cases import, NHPs and provide facilities at which NHP-based investigations can be performed. Collectively, these nonprofit and for-profit centers house several thousand NHPs, in breeding colonies and as animals on experimental protocols. Like the United States, various European countries such as Germany and The Netherlands and Asian nations such as China, Japan, and Indonesia also have centralized centers to facilitate NHP-related research. Most primate centers (for example, the US NPRCs) have detailed, computerized animal medical records including physiological and genetic data, necropsy data on animals that have died, and pedigree data going back several generations. The centers also collect and distribute biological samples, such as tissues and blood, for animals across these large pedigrees. These animal health records and associated biomaterials are a potential source for identifying animals with specific physiological phenotypes that can serve as preclinical models for human disease. Information on genotypes and molecular phenotypes, through DNA or RNA sequencing of archived tissue samples, can be obtained, even from animals that are no longer in the colony. Third, NHP research is highly regulated within the countries in which these centers are located (for a review of US programs, see Tardif et al. 2013 and of international programs, see Bayne and Morris 2012). The common goals of researchers, the primate centers, and the regulatory entities are to assure that NHPs are housed in appropriate environments, treated humanely, and used judiciously. In the United States, regulatory functions are performed by the US Department of Agriculture, the US NIH Office of Laboratory Animal Welfare, and institutional animal care and use committees (IACUCs). In parallel with these governmental entities, the private, nonprofit AAALAC International provides voluntary accreditation and assessment programs for NHP facilities.

Genetic Resources for the Rhesus Macaque (Macaca Mulatta)

Overview

The rhesus macaque is the most widely used NHP for translational research and the NHP for which there is the most information regarding genome structure and expression. Rhesus are

used in all areas of translational research (as reviewed by Capitanio and Emborg 2008; Izpisua Belmonte et al. 2015; Phillips et al. 2014). The rhesus has the widest geographic range of any NHP, from Afghanistan and Pakistan in the west, across the Indian subcontinent to south China in the east (for a map, see Rogers and Gibbs 2014). This extensive geographic distribution has lead to regional differences in genetic sequence that have developed over evolutionary time, leading to phenotypic differences in animals currently used for research. For example, Indian and Chinese-origin rhesus differ in physical and immunological parameters that can affect experiments if the two types of animals are intermingled in an experimental cohort (see Kanthaswamay et al. 2014 and references therein). Chinese-origin animals, when challenged with commonly used laboratory strains of simian immunodeficiency viruses (SIVs), develop lower plasma viral loads (Ling et al. 2002) and slower disease course (Trichel et al. 2002) compared with Indian-origin animals. These features of response to viral infection have led to the preferred use of Indian-origin rhesus for many HIV/AIDS-related experiments, for example, for vaccine development. A result has been a concomitant emphasis on developing genetic tests for the geographic origin of animals (see below) and also preferred breeding of Indian-origin animals versus either Chinese-origin animals or Indian-Chinese hybrids at some primate centers, such as the US NPRCs. However, the use of Indian- versus Chinese-origin rhesus for AIDS-related experiments has considerable nuance depending on the type of experiment being performed and the SIV stock being used (reviewed by Sui et al. 2013). For example, Chinese-origin animals exhibited the high viral loads characteristic of Indian-origin animals when the SIV strain used in the experiment was passaged in Chinese-origin animals prior to infection (Burdo et al. 2005). Furthermore, it has been suggested that Chinese-origin animals may be preferable for developing protocols for eliminating viral reservoirs (Ling et al. 2014). The main point for the present review is not the detail of experimental design and rationale for selection of Indian or Chinese-origin animals, but that rhesus used for experiments have been divided into genetic subtypes based broadly on their geographic origin and that this influences experimental design and husbandry of captive populations.

The whole genome sequence of the Indian-origin rhesus, published in 2007 (Gibbs et al. 2007), was a landmark for understanding NHP genetics and applying knowledge of the genome to translational research. This first version of the rhesus genome sequence helped catalyze experiments that have investigated the complement of rhesus genes relative to humans, gene expression in diseases as models for humans, and the relative genetic variation within animal cohorts.

This sequence was determined using shotgun Sanger fluorescent sequencing, which was the state of the art at the time. The sequence was derived primarily from a single Indian-origin animal obtained from the Southwest NPRC (San Antonio, TX) and was a draft sequence of moderate quality. As described by Rogers (2013), this draft sequence was largely complete in that it contained nearly all of the sequences of the animal but was not comprehensive enough to be continuous over distances of 10's of millions of base pairs. Thus, the draft sequence contained many gaps and those areas of sequence that are contiguous could not always be ordered relative to each other with certainty. Furthermore, as shown by Zheng et al. (2012), mistaken gene annotations occurred when the imperfect draft assembly was queried using an automated gene model pipeline. Errors in the draft sequence were expected. Additional analyses of the genome through sequencing of more animals and molecular characterization of expressed transcripts were necessary to obtain a more useful sequence.

The rhesus genome sequence assembly has been improved markedly by use of next generation (NextGen) technologies for both DNA and RNA. NextGen technologies rely on the use of highly automated, relatively inexpensive sequencing of many small fragments of nucleic acid, leading to very large collections of short fragments that can be ordered more precisely because of their quantity and redundancy. A further advance for whole-genome DNA sequencing combines use of this short-read technology with automated techniques that provide longer sequence reads, thus facilitating assembly into longer continuous regions (Rogers 2013). The NextGen concept has also been applied to sequencing of RNA. This technology, termed RNA-Seq, helped annotate the rhesus assembly by correcting errors in mRNA coding sequences, identifying mRNA isoforms not previously identified in the sequence, and beginning a characterization of noncoding RNAs that can also influence gene expression, for example, in response to infection (Peng et al. 2014). As a result of these additional functional studies, as well as enhanced informatics procedures, an improved Indian rhesus genome assembly was reported by Zimin et al. in 2014. The rhesus assembly continues to be improved by additional genome sequencing. The current version of the Indian-origin rhesus genome assembly must still be considered to be a draft sequence, but it has been improved considerably for use in translational research, for example, for constructing physical maps of the rhesus genome using single nucleotide polymorphisms (SNPs), which can be used to map disease genes, for studying gene expression in rhesus compared with humans and for designing nucleic acid probes and primers for development of genetically modified animals.

Whole-genome sequences of the Chinese-origin rhesus were reported by Fang et al. 2011 and by Yan et al. 2011 for a single male animal and a single female animal, respectively. These sequences were obtained by NextGen techniques. Both sequences were assembled by using, as scaffolds, the Indian-origin rhesus and human assemblies current at the time rather than by a de novo assembly. Yan et al. (2011) identified 25 human single copy genes that appear to be absent in rhesus macaque genomes, both Indian and Chinese. Importantly, these investigators verified the results using transcriptional and polymerase chain reaction-based assays independent of the data from which the Chinese-origin macaque assembly was derived. These results therefore are likely to reflect the actual genome sequences rather than the type of assembly artifacts noted by Zhang et al. (2012). Some of these sequence differences of macaques relative to humans could potentially be relevant to translational studies. For example, both Chinese and Indian-origin rhesus have a premature stop codon in the second exon of the opioid receptor mu1 gene, which encodes a protein that is a primary target of opioids (Yan et al. 2011).

Exome Sequencing

Sequencing of just the exomes in an experimental animal can be as much as 10-fold less expensive than sequencing the entire genome. In terms of rhesus genetic models, exome sequencing can reveal mutations in coding sequences that can cause disease and may therefore be analogous to some human Mendelian diseases that are caused by mutations in coding regions. Cornish et al. (2016) have recently demonstrated the feasibility of exome screening of rhesus. More than 95% of the

exomic sequences, isolated using a human capture array, were aligned against the rhesus whole genome assembly. As a proof of principal for identifying loss of function mutations, the investigators identified a mutation in one copy of the BchE gene (which encodes the enzyme butyrylcholinesterase) in one of the experimental animals. The animal that was heterozygous for the BchE mutation also had 30% of BchE activity in its blood, compared with an unrelated cage mate, demonstrating that the loss of function mutation influences the BCHE phenotype.

Sequence Variation

As discussed above, the first Indian- and Chinese-origin rhesus sequences were determined from single animals. It is important for translational studies to extend sequencing to many animals to understand the extent of genomic variation in the experimental population. Furthermore, it is known from candidate gene approaches that there are sequence variations in specific rhesus genes that parallel mutations in humans. Examples include a mutation associated with macular degeneration, and mutations in the serotonin transporter and mu-opioid receptor (Fawcett et al. 2011; Vallender and Miller 2013). Various investigators have begun to characterize genetic variation in Indian-origin rhesus. Fawcett et al. (2011) identified approximately 14 million potential SNPs in Indian-origin rhesus. This was accomplished by performing relatively low coverage sequence of three animals, including resequencing the animal used by Gibbs et al. (2007) for derivation of the original rhesus sequence. They also considered data available from 97 other animals. A small subset of these SNPs (approximately 4100) was analyzed as potential candidates for deleterious mutations. Of these, approximately 10% were identified as being potentially damaging. Yuan et al. (2012) took a different approach to examine the genetic diversity of Indian-origin rhesus, including a comparison with cognate genomic regions in humans. They reduced the amount of sequence to be analyzed (and thus the cost of the investigation) by considering sequences transcribed in just one tissue of both rhesus and humans (the hippocampus) in combination with DNA regions that bind histones (and therefore, which can be captured and sequenced) in 14 rhesus compared with 14 humans. Their results suggest that the rhesus is at least three times more diverse than humans, but that the frequency of mutations that can potentially have deleterious effects on protein function is more closely equivalent in humans and rhesus. Recently, Zhong et al. (2016) analyzed the sequences of 31 rhesus (26 Chinese origin and 5 Indian origin) for polymorphisms. They identified 46 million polymorphic sites in the rhesus genome. Taken together, these various studies have identified many SNPs in the genomes of both Indian- and Chinese-origin animals. In general, the rhesus sequence appears to be significantly more diverse than that of the human, even when only base changes (as opposed to indels and segmental duplications) are considered. Some of these SNPs are likely to represent potentially deleterious polymorphisms that may facilitate the development of rhesus models of specific human diseases. The several million SNPs identified in these studies have not yet been assembled into a SNP map analogous to the human HapMap, which has been used very successfully for identification of genomic regions that harbor disease genes (International HapMap consortium 2007). A “rhesus HapMap” should be of similar utility for rapidly identifying genetic regions that contain disease genes analogous to those of humans.

A more limited, but important use of rhesus SNPs, has been the development of a SNP array that can be used to distinguish Indian- from Chinese-origin animals and to identify Indian-Chinese-origin hybrids in captive colonies (Kanthaswamy et al. 2014b). These investigators designed an array that queries SNPs at 91 positions across the rhesus genome and detects Indian-Chinese hybrid rhesus that have more than 15% admixture of genes from one geographic variant or the other. This test has facilitated the maintenance of pure (within the statistical limits of the test) Indian-origin rhesus breeding colonies at the US NPRCs. Genetic tests such as described in Kanthaswamay et al. (2014b) can help minimize the variable of potential differential response to SIV infection for HIV/AIDS-related investigations using animals from these centers. A similar approach has been taken for design of a small SNP array that can be used for pedigree analysis. However, when tested with Chinese rhesus at the California NPRC, it was unclear if this array provides more accurate results than the analysis of microsatellites that has traditionally been used for this purpose (Ross et al. 2014). Likely, arrays with greater numbers of SNPs will be required for accurate pedigree analysis.

Complex Genomic Loci: The Rhesus Major Histocompatability Complex (MHC)

The MHC locus plays a central role in regulating the immune response of humans and animals to pathogens and for recognizing foreign tissue or malignant cells, which are then destroyed by cytotoxic T cells. For reviews that discuss the rhesus MHC, see Shen et al. (2013) and Wiseman et al. (2013) and references therein. The literature regarding serological methods to characterize the presence of specific proteins of the MHC is voluminous. DNA tests based on microsatellites have also been used to characterize some basic aspects of the MHC locus of animals in NHP breeding colonies (Wiseman et al. 2013 and references therein). Various investigators have demonstrated that certain alleles of the MHC locus enhance resistance to SIV infection (Loffredo et al, 2007; Mothé et al. 2002). Therefore, the particular MHC alleles harbored by the animal can affect experimental design.

Until 2004, there was no detailed knowledge of the genomic architecture of the rhesus MHC, since a complete MHC locus had not been sequenced. An aspect of the MHC locus that makes it refractory to characterization at the DNA level is its extreme complexity in regard to reiterated genes and polymorphisms among different animals. Even the best current shotgun-based whole genome approaches cannot provide an unambiguous sequence for the MHC. To solve this problem and provide a baseline sequence for the rhesus MHC, Daza-Vamenta et al. (2004) isolated a series of overlapping bacterial artificial chromosome clones that spanned the MHC of a single Indian-origin animal. They sequenced these clones to obtain a sequence across the entire rhesus MHC and also compared it with a partial sequence from a second animal. The most striking finding was that the entire MHC region of the rhesus is significantly larger than that of the human (5.3 megabases for rhesus, 3.7 megabases for human). The MHC is classically divided into three regions, termed Classes I, II, and III. Class I and II genes are central to control of immune responses. The difference in size between the rhesus and human MHC loci is largely due to an increased number of genes within the rhesus MHC Class I region in rhesus relative to humans (22 genes in rhesus versus six in humans). This difference in gene content

in the MHC Class I region appears to reflect an increase in potentially functional genes, and not pseudogenes.

The complexity of the rhesus MHC locus, as described by the investigations of Daza-Vimenta et al. (2004), means that it is necessary to have facile means of characterizing the MHC of the many rhesus that are used as experimental subjects, for example, for development of vaccines. An important advance was made by the laboratory of Dr. David O'Connor at the Wisconsin NPRC and his colleagues, as summarized by Wiseman et al. (2013). These investigators have developed PCR-based assays that employ primer pairs that bind highly conserved sites that flank the polymorphic sites within MHC class I transcripts. The repertoire of expressed MHC alleles and their relative abundance can be obtained for any given animal when these PCR products are sequenced. Following demonstration of the utility of these procedures in 2009 (Wiseman et al. 2009) throughput has been increased and cost has been decreased by use of improved NextGen sequencing platforms, as they have been commercialized over time. This sequencing strategy has also been applied to genomic DNA, making it possible to obtain information about the MHC genotype, as well as the MHC phenotype. Taken together, the full genomic characterization as demonstrated by the investigations of Daza-Vimenta et al. (2004) and high throughput PCR-based sequencing as described by Wiseman et al. (2013) make it feasible to characterize experimental animals in terms of MHC gene expression before they are assigned to a protocol, thus reducing the potential variability of immune responses as a confounding factor in a given experiment.

Transcriptomics

The transcriptome is defined as the complete set of transcripts in a cell, including their quantity (Wang et al. 2009). Comparison of the transcriptome of an animal model with that of humans in various tissues and in diseased and normal states is an important tool for translational research. Microarrays commercialized by Affymetrix (http://www.affymetrix.com) and Agilent Laboratories (http://www.agilent.com), developed in collaboration with academic researchers (Duan et al. 2007; Spindel et al. 2005; Wallace et al. 2007) provided the first assay systems that facilitated a relatively comprehensive analysis of the rhesus transcriptome. These arrays allow investigators to query more than 45,000 rhesus sequences and have been used in a number of translational studies, for example in the field of infectious disease research (Palermo et al. 2013), including HIV/AIDS (Peng et al. 2014).

Microarray technology is relatively inexpensive and allows high throughput. However, because the technology is based on the hybridization of fluorescently labeled DNA copies of cellular RNA with probes on the microarray chimp, there are limitations in regard to dynamic range and signal to noise. The more modern method of RNA-Seq, which involves NextGen sequencing of DNA copies of cellular RNAs, can obviate these issues and provides additional information, for example, by identifying nucleotide polymorphisms in related RNA transcripts (Wang et al. 2009; Palermo et al. 2013). RNA-Seq is being used by many investigators to study aspects of gene expression in rhesus models of human disease. The investigations reported by Sureschshandra et al. (2016) are a recent example. This study identified changes in gene expression in female macaques that exhibit high levels of voluntary alcohol administration, an animal model for alcohol use disorder in humans. The investigators found differences in gene expression that can influence conditions found in human alcohol use disorder, including higher incidence of infection, delay in wound healing, and increase in cardiovascular disease.

Genetic Resources for the Cynomolgus Macaque (*Macaca fascicularis*)

Overview

Like the rhesus, the cynomolgus macaque (CM; *Macaca fascicularis*) has been used for many different types of investigations, although in the United States it is used sparingly for HIV/AIDS-related studies. CMs are also sometimes called crab eating macaques or long tailed macaques and occur as wild populations in southern Asia, Indonesia, the Philippines, and the Indian Ocean island of Mauritius. Due to their wide geographic distribution and introgression with rhesus, CMs can harbor significant genetic diversity (Eberling et al. 2011; Kanthaswamay et al. 2014). This diversity, in analogy with Indian- and Chinese-origin rhesus, can potentially influence experimental design and outcomes. Mauritian CMs, which were derived from a small number of founder animals introduced by European seafarers 400 to 500 years ago, provide cohorts of animals with much more limited genetic diversity. In particular, Mauritian CMs exhibit very limited MHC diversity, facilitating HIV/AIDS-related investigations in which animal to animal variation in the MHC is not a confounding variable (reviewed by Wiseman et al. 2013).

CMs are the most commonly used NHPs for testing the effects of pharmaceuticals on physiological parameters and for testing toxicity and pharmacokinetics. Colman (2016) cites two specific examples in which the response of individual CMs to a small molecular therapeutic or an antibody-based therapeutic, respectively, depended on the geographic origin of the animals used in the studies. As also noted by Colman (2016), a cohort of CMs used in a given study can often include animals of different geographic origins, thus contributing to experimental variability. Although some variation can occur as a result of the microbes to which animals are exposed in different captive colonies (Colman 2016), it would be expected that genetic differences can also play a significant role. The issue of variability emphasizes the need to characterize CM genetic diversity as it affects drug testing and has motivated whole genome sequencing of this species.

The Whole Genome Sequence and Sequence Variation

Three research groups have reported whole genome sequencing data on CMs. All three sequences were assembled using the rhesus and human assemblies as scaffolds. Therefore, there is, as yet, no de novo assembly of the CM genome. Taken together, these three studies form a basis for characterizing genetic differences in CMs of diverse geographic origin that are likely to be highly relevant for improving the use of this animal model in translational research, and, in particular, in drug development.

Ebeling et al. (2011) sequenced a female CM from Mauritius. This approach allowed for many comparisons with the rhesus and human sequences, including identification of polymorphisms in orthologus genes between CMs and rhesus and CMs and humans. Using both gene sequences and expression arrays derived from them, Ebeling et al. (2011) also compared gene structure and expression in CMs related to two gene families that are involved in drug metabolism in humans and macaques (Cytochrome P450 and Solute Carriers for Organic Ions). Yan et al. (2011) sequenced a female CM of Vietnamese origin. These investigators report that a comparative analysis of CM sequences with those of the human suggest a high degree of

similarity, including genes encoding proteins with domains that interact with drugs, the "drugable genome." Yan et al. (2011) found 19 genes, identified as within the human drugable genome, that appear to be pseudogenes in CM. Higashino et al. (2012) sequenced a male CM of Malaysian origin. Analysis of the data emphasizes discovery of single nucleotide differences between, respectively, the Malaysian CM and Indian-origin rhesus and the Malaysian CM and the Vietnamese CM sequenced by Yan et al. (2011). Higashino et al. (2012) identified more than 8 million single nucleotide variants in these analyses.

Transcriptomics

The analysis of Ebeling et al. (2011) included expression analysis using oligonucleotide arrays produced in their laboratory. More recently, Huh et al. (2012) and Lee et al. (2014) have used RNA-Seq to comprehensively annotate the CM transcriptome. This annotation will facilitate many studies using RNA-Seq, for which investigators can now identify transcripts, which change in abundance under various experimental conditions.

Genetic Resources for the Vervet Monkey (*Chlorocebus aethiops*)

Overview

Monkeys of the genus *Chlorocebus* (vervets, sometimes called African green monkeys) have wide ranges in Africa and were also introduced by seafarers to the Caribbean islands of St. Kitts, Nevis, and Barbados. Although vervets are used for a variety of studies, for example, neurodegeneration (summarized in Jasinska et al. 2013), a major interest from a biomedical perspective is that they are natural hosts for SIV. When infected with the virus, vervets do not develop AIDS. Aspects of the physiological factors associated with resistance to SIV can be studied in both wild and captive populations (Ma et al. 2014).

The use of the term vervet versus African Green monkey can be confusing, and the two are sometimes used interchangeably. This review will discuss the vervet subspecies *C. aethiops sabaeus*, which is used most commonly in translational studies, due in large part to its availability from captive colonies from primate centers in the Caribbean and from the US Vervet Research Resource at Wake Forest University.

The Whole Genome Sequence and Transcriptomics

The genome sequence of *C. aethiops sabaeus* was reported in 2015 (Warren et al. 2015). These investigators assembled the sequence de novo (i.e., did not use the rhesus or human sequence as a scaffold). In addition, they annotated the genome by RNA-Seq analysis of transcripts from several tissues, characterized segmental duplications in different animals, and sequenced the MHC of the animal from which the basic whole genome sequence was derived. Warren et al. (2015) therefore represents one of the most comprehensive genome analyses of a NHP species reported in a single study. Huang et al. (2015) developed a SNP map based on sequencing 721 animals from the Vervet Research Resource, which will be useful for various types of genetic analysis. The sequencing studies cited above, together with extensive phenotype data regarding brain structure, behavior, cognition, metabolism, and diet (Jasinska et al. 2013), position the vervet for wider use for translational studies.

Genetic Resources for the Baboon (Genus *Papio*)

Overview

Baboons of the genus *Papio* have been used for many years for a variety of translational investigations, including studies of cardiovascular disease, obesity, bone disorders, and intrauterine grown restriction (reviewed by Cox et al. 2013), as well as female reproduction (Bauer 2015) and xenotransplantation (Schuurman 2016). In some cases, different species or subspecies of baboons have been used for various studies, and these are not always well specified in the literature. Many translational investigations have been carried out using the large colony of pedigreed baboons housed at the Southwest NPRC (Cox et al. 2013). Founders of this pedigree comprised olive baboons (*Papio hamadryas Anubis*), yellow baboons (*P. hamadryas Cynocephalis*), and hybrids of the two subspecies. Various species of baboons have often been studied in the wild, particularly in regard to infectious disease (see for example, Tung et al. 2009).

The Whole Genome Sequence and Other Genetic Resources

The whole genome sequence of the baboon has not yet been published, although a draft sequence can be accessed through public databases (see for example, Cox et al. 2013; Wall et al. 2016). In the absence of a published high-quality draft sequence, much of the genetic analysis using the baboon as a preclinical model has been performed using a genetic linkage map (Cox et al. 2006; results summarized in Cox et al. 2013). These studies have concentrated on identifying quantitative trait loci, regions of the genome that contain genes influencing common human conditions with high genetic complexity, such as hypertension, cardiovascular disease, and obesity. The specific genes within the quantitative trait loci that influence the condition can be determined by additional techniques, such as comparative RNA analysis of animals with different phenotypes (Karere et al. 2013).

Genetic Resources for the Marmoset (*Callithrix jaccus*)

Overview

The marmoset is a small, new world NHP that has several novel features relative to the old world NHPs discussed above. Marmosets are much smaller than macaques or baboons, reach sexual maturity much faster, and routinely give birth twice a year, usually to nonidentical twins. These features favor marmosets as subjects for derivation of genetically modified animals (Izpisua Belmonte et al. 2015; Kishi et al. 2014; Sasaki et al. 2009), despite their greater evolutionary distance from humans relative to old world monkeys. Marmosets are much shorter lived than macaques and exhibit many age-related changes over relatively short time frames (the average life span is 5–7 years) that mirror those of humans, including beta amyloid deposition in the cerebral cortex; metabolic conditions such as diabetes and renal disease; and cancer (reviewed in Tardif et al. 2011). Thus, the marmoset is a useful animal model to study various aspects of aging as well as other aspects of human disease.

The Whole Genome Sequence

The genome sequence of the marmoset, obtained from an animal from the Southwest NPRC, was reported in 2014

(Marmoset Sequencing and Analysis Consortium 2014). This sequence was derived from Sanger-based techniques, with some contribution from NextGen technologies. Partial sequence was also obtained from nine other animals (all from captive colonies in the United States) in order to characterize common polymorphisms. Analysis of the sequence identified several candidate genes that may have been involved in the evolution of some of the unique features of the marmoset, including small body size and twinning. Following publication of this draft sequence, Sato et al. (2015a, 2015b) performed resequencing using NextGen technologies. The animal that was sequenced was from the Central Institute for Experimental Animals in Japan. These data have been used to improve the marmoset genome assembly.

Transcriptomics

Maudhoo et al. (2014) sequenced RNA isolated from 5 different tissues using RNA-Seq and annotated about 51,000 transcripts from more than 10 thousand genes. Shimizu et al. (2014) sequenced RNA from four different tissues isolated from six animals. These investigators identified approximately 48,000 transcripts, including those encoding Cytochrome P450s and flavine containing monooxygenases. These findings begin to position the marmoset as a potential model for drug development, akin to CMs.

Conclusions

The genomic resources available for the five NHPs most commonly used for translational research are beginning to catch up with the wide, historic use of these animals in human-related studies. The research community can expect these genetic resources to be used increasingly in translational science, particularly as whole genome sequencing and RNA-Seq are used to characterize more animals and thus refine knowledge about NHP genomes. Genomic resources are particularly advanced for the macaques (rhesus and CM), both of which have well-characterized whole genome sequences and transcriptomes. The research community can now expect that this genomic information will be used increasingly to help develop new animal models, explain and enhance results already obtained, and aid in stratifying experimental animal cohorts to enhance reproducibility.

Three emergent areas (among many) appear likely in the near term to synergize with the genetic information that is available for NHPs and therefore impact translational research.

First, the high throughput technologies already used for genomic characterization are likely to be employed to an even greater extent to identify new NHP models within the colonies that are housed at the various primate centers around the world. Larger numbers of animals are likely to be sequenced, thus identifying potentially deleterious mutations, present in the heterozygous state in various breeders, particularly for the development of models for Mendelian disorders. Animals homozygous for a deleterious mutation can be bred from existing stock, thereby generating enough animals to test a given model. Second, more animal models are likely to be produced by genetic engineering. Very significant progress has been made in producing genetically engineered macaques and marmosets. These investigations include development of monkey models for Huntington's disease (Chan 2013), diseases caused by mitochondrial mutations (Tachibana et al. 2009), and autism (Liu et al. 2016). Third, other emerging technologies will be applied to NHPs to develop new models that are closer to humans than rodent models. An example of this is analysis of the microbiome, which has been used to examine the response of NHP preclinical models to vaccines for HIV/AIDs (Handley et al. 2012, 2016).

In summary, the research community can expect further development and use of NHP models to facilitate translational science, particularly as the current foundations of NHP genomics is enhanced and expanded.

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