Monkeying with monkey genes

Advances in genome-editing techniques have enabled researchers to create specific genetic modifications in laboratory animals such as mice, rats, fruit flies and zebrafish. This is often done to create models of human diseases that can be studied to learn about their etiology, pathology and mechanisms and to test potential therapeutic strategies. But many human diseases can be appropriately modeled only in primates, and, until recently, genome editing in primates has been largely unsuccessful as well as prohibitively expensive, with the only reported successes in generating transgenic primates achieved by using the technique of virus-mediated gene transfer.

The viral vector strategy lacks the efficiency and precision required for broad utility, however. Now, researchers led by Weizhi Ji of the Yunnan Key Laboratory of Primate Biomedical Research (Kunming, China) have successfully applied two advanced genome-editing techniques in monkeys, producing cynomolgus macaques with targeted genomic modifications.

The first technique, dubbed the CRISPR/Cas9 system, uses short RNA sequences to direct gene editing, enabling alterations at the single-base-pair level. Ji’s team disrupted two target genes simultaneously in macaque embryos by co-injecting the embryos with Cas9 mRNA and guiding RNA. The embryos were transferred to surrogate female macaques, resulting in 10 pregnancies, one of which was later miscarried. At the time the report was published, one female had delivered twin female offspring at full term by caesarean section and the remaining eight females were mid-gestation. Preliminary analyses of tissues from the infant monkeys showed that the CRISPR/Cas9 system resulted in site-specific gene modification with no off-target effects (Cell 156, 836–843; 2014). Further analyses will be forthcoming when the remaining infants are delivered and when the transgenic monkeys reach adulthood.

Ji’s team also investigated the use of the TALEN system in editing macaque genomes. This technique uses special nucleases to bind and cut DNA at a specified genomic location, creating specific mutations. In this case, the targeted gene encodes methyl-CpG binding protein 2 (MECP2), mutations of which are associated with Rett syndrome, an X-linked disorder that affects human females. Six surrogate females became pregnant after transfer of modified embryos, and one female infant was born and had reached 4 months of age at the time the report was published (Cell Stem Cell, 14, 1–6; 2014). Successful MECP2 mutagenesis with no off-target effects was reported.

Together, the studies’ results show that in vivo genome editing is possible in nonhuman primates, which may enable the development of better models of human diseases.

Monica Harrington

LIFESPANS PREDICTED IN A FLASH

A new study suggests that an organism’s lifespan is predictable at early adulthood by looking at the activity of its cells’ mitochondria. Mitochondria are the organelles that supply energy to the cell. During energy production, mitochondria produce reactive oxygen molecules, such as free radicals, that can cause stress and damage the mitochondria. It was recently discovered that mitochondria sometimes produce these reactive oxygen molecules in short, repeated bursts: these are called mitoflashes.

Meng-Quí Dong and his colleagues at the National Institute of Biological Sciences in Beijing, China, visualized the mitoflashes produced by mitochondria in Caenorhabditis elegans by targeting the energy-producing organelles with a fluorescent protein that glows yellow when viewed under a microscope. In regular worms, two mitoflash bursts occurred: one during the worms’ egg-laying period in early adulthood and one during senescence.

The team compared mitoflash rates in two mutant C. elegans strains, one with a shorter lifespan, lasting 21.1 d on average, and the other with a longer lifespan, lasting 30 d or more on average. Remarkably, the pace of mitoflashing in the first mitoflash burst was found to correlate with lifespan in the worms. The shorter-lived worms had more frequent mitoflashes than the longer-lived worms (Nature doi:10.1038/nature13012; published online 12 February 2014).

Recognizing that aging is determined not only by genetic factors but also by environmental factors, the researchers next gave different groups of worms various treatments that have been shown to alter the lifespan of C. elegans. Conditions that extend the lifespan of the worms, such as exposure to heat shock or starvation, resulted in less frequent mitoflashes during the early-adulthood burst. And conditions that reduce the lifespan of the worms, such as treatment with substances that increase production of reactive oxygen molecules, resulted in more frequent mitoflashes. These results suggest that the bursts of activity in the mitochondria of the worm’s cells can accurately predict how long the worm will live. “Mitochondrial flashes have an amazing power to predict the remaining lifespan in animals,” Dong told Nature News.

A common theory suggests that mitochondria are the biological clock that drives aging. In addition to supplying energy to cells, mitochondria are also involved in cell death and the control of the cell cycle and cell growth. The findings of this study certainly support that theory by demonstrating the intricate link between mitochondrial activity and lifespan. Said Dong, “There is truth in the mitochondrial theory of aging.”

Kara Rosania
Using privately owned animals in a study of human subjects

Julie Schnepps, a private-practice veterinarian in California, was interested in the human-animal bond, and an opportunity was emerging for her to further that interest. One of Schnepps’s clients, Dr. Lucas Roman, was a reconstructive surgeon on a sabbatical leave from Great Eastern University. He suggested to her that it might be of importance to determine if pet-assisted therapy could lower the level of anxiety that many of his teenage patients experienced after undergoing surgery for traumatic facial injuries. Schnepps jumped at the chance but didn’t know how or where to begin, so she recruited the help of Dr. Maria Torres, her friend and a laboratory animal veterinarian at Riverbank University, a nearby research university. Schnepps and Torres decided that measuring blood cortisol levels in adolescent patients that did or did not have pet dogs would provide the initial data they needed. As a favor to Torres, Riverbank’s clinical laboratory agreed to carry out the cortisol analyses at a minimal cost, which Schnepps would pay using funds from her private practice. Torres did not think that IACUC approval was necessary for the study.

Roman was a faculty member at the Great Eastern University medical school, and his surgical practice was at the Great Eastern Hospital. The hospital and the university were legally separate entities, although medical school students used the hospital for part of their training. Roman received approval for the study from the hospital’s Human Research Subjects Committee (analogous to an IACUC) and its Infection Control Committee. Once his patients were postoperatively stable, healing well, yet still hospitalized, they would be brought to a special visiting room where they would be allowed a short, controlled interaction with their pet dog. Blood samples would be collected from the patients before and after the dog visits, and then, after their discharge from the hospital, a blood sample would be collected every time they returned for a postsurgical visit.

The primary interest of Schnepps and Roman was patient stress, as reflected in cortisol levels. They hoped to demonstrate that the teenage patients who had dogs had significantly lower cortisol levels than those without dogs. Given the many variables present, this was meant to be a pilot study that might (or might not) provide a justification for moving forward with more involved research on pet-assisted therapy.

Because the dogs are such a critical part of the study, is IACUC approval needed? If so, which IACUC should review the protocol: Riverbank University’s or Great Eastern University’s? Roman is a faculty member at Great Eastern University’s medical school, as part of their training, medical school students might observe the planned patient-animal interaction on video monitors. Does this fact affect the need for IACUC approval?

RESPONSE

Patient-pet interactions

Barbara C. Hansen, PhD

The described scenario has several aspects. The first is the desire of a private veterinarian to measure cortisol levels in blood samples from pet owners and from people who don’t own pets. Though well-intentioned, this notion does not constitute a valid research study of stress levels in adolescent patients who have or do not have pets. There is no need for any IACUC review of this aspect.

The second aspect is the sampling of blood in adolescents before and after a short visit by their own pets in a special room in the hospital and during their postoperative recovery period. Approval of this study by the university’s human use committee implies that all concerns related to the inclusion of humans in the study, such as infection risk, dander risk and room sanitation, were addressed to the committee’s satisfaction. In my opinion, this aspect of the scenario also does not constitute a well-designed research study of human stress (or even a well-designed pilot study), but if it satisfied the human use committee, then so be it. There is no need for IACUC review of this aspect.

The third aspect is the recording of patient interactions with their own pets (assumedly with the patients’ knowledge and consent) and the possibility that medical students might view the video. In my opinion, this does not require IACUC approval for multiple reasons. The patients and their parents or legal guardians have presumably agreed to participate in the study with full knowledge of the patient-pet meeting conditions (including the video). There is no animal welfare issue of any sort involved in the scenario provided. There is no research done on the animals, nor will any conclusions regarding the animals be derived. Given the information provided, there is no abuse or risk to the pets themselves beyond normal human-pet interactions. The study is on patients’ responses to their own pets.

I see no reason for any IACUC involvement here. If there were an IACUC issue here, however, the only IACUC implicated is the one at Great Eastern University.

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Pet-assisted therapy

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At first review of this scenario, our impression was that it did not require IACUC approval. The scenario describes the use of privately owned pets for research on human subjects in a hospital setting. One must remember that the research subject of this study is the human patient—not the patient’s pet dog. The pilot investigation does not include any animal research, teaching or testing, and neither institution (academic or hospital) has ownership of the animals. In addition, this study is not currently supported by funding from the Public Health Service (PHS). The scenario did not indicate whether any of the entities involved are accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) International.

Ownership and funding as related to the need for animal program oversight is addressed by AAALAC International (https://www.aaalac.org/accreditation/faq_landing.cfm#A1) and by PHS guidance1. “The PHS Policy covers live vertebrate animals used or intended for use in research, research training, and biological testing activities conducted or supported by the PHS. The PHS Policy and the Animal Welfare Act and Regulations (AWAR) do not distinguish between animals owned by the institution and privately owned animals. Pets used in research must be covered under an IACUC-approved protocol. The institution must have an OLAW-approved Animal Welfare Assurance covering all performance sites. The institution should ensure that the informed consent of the owner is obtained prior to the conduct of the research. The institution may want to involve their legal counsel in the development of informed consent documents.”

Whether the proposed activities as described are covered may hinge on the Letter of Assurance for the institutions involved in this scenario. Some institutions have broad letters of assurance and state that all animal activity at that institution is covered. Therefore, IACUC involvement may be warranted2,3. Furthermore, if the goal of the study is to generate pilot data that may be used for PHS-funded projects in the future, as suggested in the scenario, then IACUC review might be necessary. If future studies involve institutionally owned animals, then oversight is absolutely required.

1. Animal Welfare Act as Amended (7 USC 2143).

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the activities involving animals occur should take precedence. In accordance with PHS guidelines on collaborations, a memorandum of understanding or a service agreement should be used to clarify regulatory oversight and study and grant funding management responsibilities among the collaborating entities.

A confounding factor is Roman's sabbatical leave. Whether faculty members who are on leave are able to represent the institution and oversee research involving humans or animals is typically governed by institutional policy. Some institutions do not allow faculty members who are on sabbatical leave to be the responsible party for such research.

An additional confounding factor is the inclusion of medical student training as a potential option. We feel that this option would be considered animal use in medical training. Therefore, if this aspect is pursued, we feel that it requires IACUC protocol review, review of occupational health and understanding of the applicable regulations, regardless of who owns the animals.

Finally, we feel that the dogs that participate in this study should complete programs for certification as hospital therapy dogs before this study goes forward. It was prudent that the hospital's human subjects and infection control committees reviewed and approved this activity, but we believe that there may be other committees that should be consulted for approval as warranted by the institution's policies. These include the hospital's risk management office, board of directors, legal counsel and environmental health and safety office. We also suggest that the investigators confer with the institutional veterinarian and review the hospital health clearance guidelines used by national pet-assisted therapy organizations (e.g., https://www.avma.org/KB/Policies/Pages/Guidelines-for-Animal-Assisted-Activity-Animal-Assisted-Therapy-and-Resident-Animal-Programs.aspx).


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RESPONSE

Better safe than sorry

Adrienne Ferguson, BAS, RVT, LATG, CMAR & Katherine A. Naff, DVM, DACLAM, CPIA

The dogs in this scenario would be involved in a human subjects research project. The only costs are those related to blood cortisol testing, which will be covered by private funding; therefore, the provisions of the Public Health Service (PHS) Policy on Humane Care and Use of Laboratory Animals need not apply. If strict interpretations of the Animal Welfare Act (AWA) definitions of 'animal' and 'research facility' are used, however, then IACUC approval of this project may be necessary. The AWA defines an animal as "any...warm-blooded animal...used for research." Great Eastern Hospital meets the AWA definition of a research facility in that it is using dogs as a study component and presumably receives some federal funding for the conduct of its institutional research program. In addition, the US Department of Agriculture holds research institutions accountable for review and approval of proposed activities related to the care and use of animals and delegates the authority for oversight of animal care to the IACUC. Although the activities described seem to be innocuous, the animals are an integral part of the study, for without the dogs, there would be no patient data to collect. Even though the dogs are not experiencing pain or distress under the study conditions, they are being subjected to novel, potentially stressful activities outside their normal routine, under the auspices of Great Eastern Hospital. Injury or escape of an animal could draw negative publicity, and the lack of animal use committee oversight could further damage public perception of the Hospital and, by association, the University. For these reasons, we believe that having an animal use protocol is the best course of action for protecting Great Eastern Hospital and the University against research risks associated with the use of animals in the conduct of the study.

If an animal use protocol is necessary, then which institution should hold the protocol and provide oversight of the animal-based portion of the work: Riverbank University, Great Eastern Hospital or Great Eastern University? In our opinion, Riverbank University has no oversight responsibility; its only role is the provision of laboratory analysis on a fee-for-service basis. If Great Eastern Hospital has its own animal care program, then Roman, as principal investigator, should submit the protocol through the hospital's IACUC; this would be the simplest solution given that this is the site of the patient-pet interactions. If the hospital doesn't have an animal research program (as seems likely), however, then Roman should submit a protocol through Great Eastern University's IACUC, in which the room where patient-pet interactions occur is designated as an off-site location. Because the hospital and university are legally separate entities, the shared responsibility for animal oversight should be clarified via a memorandum of understanding (MOU). Specific details that should be delineated in the MOU include on-site responsibility for care and handling of the dogs, assignment of responsibility for occupational health, veterinary care and requirements for site visits by the University's IACUC and other regulatory or accrediting agencies. Great Eastern's IACUC should also determine whether the activities at the hospital will be considered separate from its PHS-supported activities and should state this exception in its Assurance.


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