PEER REVIEWED

The Impact of the CRISPR Revolution on Animal Healthcare

The Link Between Human and Animal Research

There has always been a link between human and animal disease study, going back to the early stories about Edward Jenner and the subsequent development of a human smallpox vaccine. For many years, researchers have developed animal models of human disease in hopes of elucidating pathways to help cure and prevent disease in man. Both humans and animals share a common enemy in bacterial and viral infections, and strategies for dealing with these are often based on the use of similar, if not identical, drug treatments. Many non-infectious diseases, like cardiac and respiratory disease, are also studied through the use of animal models as there are physical characteristics shared between man and non-human animals which offer hope of finding a better path forward in fighting and preventing disease.

Historically there has been a regulatory requirement that any new human drug be tested in a relevant animal model (at least for safety) before it could be administered to a human. If the model indicated some degree of efficacy it could lead to positive regulatory review and was often used in driving necessary investment for further development. This pushed research into different species for a variety of reasons. Pigs, for example, became a primary species for wound-healing and cardiac-related studies due to the similarities between the pig and human tissues and organs involved. Drugs developed and tested in one species could often come to be used in treating the animal for related diseases or symptoms. This practice continues to some degree today, but against a constant backdrop of 'return on investment' the work on finding new drugs specifically for animals is challenging.

The Genomic Revolution

In 2001, the first full human genome sequence was published [1]. It was a towering achievement made possible through coordination by a worldwide consortium of laboratories and individual scientists. In order to realise this lofty goal, significant advances were required not only in biological techniques, but also in the fields of high-tech machinery (robotics, sequencers) and computing resources (memory, storage, processors, and algorithms to analyse the data). The genomic revolution, as it was called, came at a significant cost (although slightly under budget) of roughly $2.7 billion[2]. What emerged was more than just a single human sequence – rather a platform to examine the life code of every species on the planet.

Not long after this, genome efforts were established for other species, resulting in sequences for the chicken (2004), dog (2005), cat (2007), horse (2007), cow (2009), and pig (2009) becoming widely available. At last check, the National Center for Biotechnology Information (NCBI) website showed that over 9000 eukaryote species (higher organisms, including humans, plants, and animals), 210,000 bacterial species, and over 32,000 viral strains have had their genomes fully sequenced[3]. While having a generic 'dog genome' available would be a boon to dog health researchers, we all recognise the substantial differences between a chihuahua and a mastiff. This first dog genome was simply the starting point, and researchers now tend to focus on the small differences within a single species to highlight susceptibility or resistance to disease. All of this too comes at a cost, but thanks to advancements in technology, we now see even these costs coming within the reach of most researchers. The '$100 genome' has long been the mantra of the genome community and we stand close to that figure today. For $350 one can get an individual dog's full genome sequence from at least one commercial organisation.

The Birth of Genome Editing

With about 23,000 separate genes identified in the human genome (the water flea wins the award for the most genes with roughly 31,000) researchers began to look for ways to determine the role each gene played in shaping life and whether it played a part in preventing or spreading disease. Clearly the most straightforward approach would be to simply remove each gene individually and look for what effect that had. In the theoretical world of laboratory science this was the perfect control, but except for the ability to manipulate the genomes of mice, this was only a pipe dream. There was some historical precedence to think it might one day be possible. Back in 1994, Dr Maria Jasin and her colleagues at Memorial Sloane Kettering published work showing that when a double strand DNA cut was introduced into the chromosome of a living cell, the cell could repair that cut and add new DNA at the cut site[4]. The problem was that Jasin's cell had been artificially created to include the recognition site for an introduced endonuclease, and she couldn't simply target the cut anywhere she wanted. It was about a decade later that Dr Matthew Porteus, working in the laboratory of Dr David Baltimore at Caltech, showed that he could combine a protein that targeted specific DNA sequences with an enzyme that cut DNA and introduce a cut at a site of his choosing[5]. These hybrid proteins were known as zinc finger nucleases (ZFNs) and Porteus demonstrated that they worked in living mammalian cells. Others were quick to show that these same proteins could be used in animal embryos to modify the genes of subsequent offspring. Genome manipulation was no longer restricted to mice.

The tool to explore the vast accumulation of DNA information now existed, but the challenge was more one of practicality and cost as each ZFN required a reasonably high degree of design knowledge and testing to find suitably specific proteins that targeted the desired site. Research progressed on targeting known genes, but due to time and cost constraints, most of the work focused on human gene targets where the payback justified the investment. Fast forward several years and a second cutting system emerged based on a protein derived from a plant-invading bacteria. Known as TALENs (Transcription Activator-Like Effector Nucleases), these proteins were larger than ZFNs but simpler to design. However as with ZFNs, construction and validation of TALENs would still take several weeks and thus prove relatively expensive. Still, research using genome editing continued on a slightly wider basis. In 2012 a third system was introduced to the scientific world, deriving from the labs of Dr Emmanuelle Charpentier and Dr

Jennifer Doudna and based on a bacterial immune system[6]. Known as CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats), it turned out to be a highly efficient method of targeting and cutting DNA. The beauty of CRISPR, aside from its inherently high cutting efficiency, is that it is so simple to design that no special knowledge is needed to order the simple components required to make it work. The components are widely available and cheap to obtain, opening up the world of gene editing to virtually anyone. This includes the livestock and veterinary healthcare fields where prior incarnations of genome editing were either too complicated or simply too expensive to implement.

Reverse Engineering the Human/Animal Link

In the years since the first genome editing technology was introduced, advances on their application on the human therapeutic side have occurred. Human gene and cell therapy treatments based on the use of ZFNs, TALENs, and CRISPR have advanced into the clinic. Targeting monogenic diseases like sickle cell anaemia and beta-thalassemia, where a permanent change in a small region of DNA can bring therapeutic benefit, has become reality. Engineering of personalised therapies to fight cancer are also in the works. In a recent controversial application of CRISPR in humans, a scientist in China claims to have edited two babies prior to birth to introduce a genetic change that would render them resistant to HIV infection. This has given rise to endless ethical discussions and the scientist himself has been censured by Chinese authorities and most of the world. But the prospect of using CRISPR this way does bring up a tantalising paradigm... what if genome editing could be used to prevent infectious disease that poses a serious threat to commercial livestock? How does that weigh against arguments regarding creation of genetically modified organisms (GMOs)? (We'll leave aside for now the possible distinction between certain modifications not being characterised as GMO.)

The Livestock Paradigm

Genetic modification to livestock can generally be considered to have an outcome that falls into one of three main categories: 1) improved health/survival; 2) safety and improved quality of life; or 3) product quality or production trait improvements.

Examples from the first category of applications, that would impact the health and survival of the animals, include African swine fever and porcine reproductive and respiratory disease. China currently maintains more than half of the world's pig population, and globally exports $20 billion in pork products annually. African swine fever is predicted to decimate 25%–35% of the Chinese swine population in 2019, which is more than the population of pigs in the US and Europe combined[7]. These are daunting numbers that will have both humanitarian and economic impacts. CRISPR could possibly be implemented to reduce the impact of this event or even entirely prevent it in the future.

Porcine reproductive and respiratory disease (PRRS) costs the US pig industry $650 million per year and $150 million per year in the UK. The avian flu pandemic of 2014/2015 cost the US $879 million[8]. There are numerous diseases like this that have the potential to be ameliorated (if not eliminated) through genetic modification. The target genes for modification by CRISPR for these specific diseases may not currently be known, but there is certainly ongoing research into routes of transmission, and factors required for productive virus infection. We know that vaccines are sometimes ineffective due to similarities of the infectious agent to endogenous proteins. Changes to some of these endogenous proteins without significant side-effects might therefore render these animals capable of mounting an effective immune response themselves.

An example of the second category of modification would be something along the lines of Recombinetics' hornless cow trait. Farmers routinely remove (or prevent from growing) the horns as a matter of safety for the farmers as well as other cattle. Eliminating the need to remove the horns would save the cow from undue stress and suffering, while providing a safer environment for other cattle. Farmers are left with a number of options to deal with this issue and so economics and public perception will come to play a major role in the adoption of genome editing in this setting.

The third category of modification is aimed at improving the quality of the end product, whether it be healthier meat, greater yield per animal, or an improved textile property. An example that presents multiple issues is beef production in Brazil and the impact this may be having on the climate. Brazil's cattle herd is twice the size of that in the US and is also the world's largest producer of meat-related emissions (methane), however the average carcass weight in Brazil is 500lbs at three years of age (compared to 800 lbs at 18–22 months in the US)[9]. The problem is that Angus beef raised in the US cannot tolerate the Brazilian heat, so these higher-producing cattle cannot currently be adopted into the Brazilian herd.

CRISPR could be used to combine the most desirable traits from each cattle, introducing key Angus traits into the Brazilian herd or genetically adapting Angus to a warmer climate, in a more efficient and precise way than traditional cross-breeding. For example, the "slick" mutation (identified in Senepol cattle in the Caribbean) which is responsible for producing shorter, slicker hair and contributing to a lower internal temperature[10] is one of the targets that Recombinetics has identified as a potential candidate for introduction into Angus beef. This modification could have a positive impact on the quality of the meat produced in Brazil (Angus beef sells for a 50% premium over Brazilian Nelore beef) and could contribute to reducing the cattle-related emissions in Brazil, providing a wider global benefit. The basis for making modifications in this third category will be driven almost completely by economics and the willingness of the markets to accept this product.

Veterinary Animal Healthcare Applications

While livestock applications always invoke the potential for discussions around GMO and food, the application of CRISPR in companion animal healthcare would appear to be much simpler and more straightforward.

Cancer is the number one killer of dogs, with 4.2 million new cancer diagnoses made each year[11], and a similar number in cats. Canines suffer from lymphoma, breast cancer, osteosarcomas, and soft tissue sarcomas, and the progression of the diseases in humans and animals follow a similar path, which has enabled researchers to develop nearly identical treatment options. Additionally, many of the same genes are involved in cancer in both humans and dogs.

One of the more recent advances in human healthcare, where CRISPR is being actively applied, is the development of CAR-T, or chimeric antigen receptor T-cell therapies, to provide a boost to the immune system to clear cancerous cells. Dogs are being used in human preclinical trials, providing some important information for direct application in animals. A small start-up in Minnesota (LEAH labs) is one vet-focused group using CRISPR in a CAR-T approach to

treat dogs with cancer. An advantage in this area is that pet owners are generally not reluctant to allow their animals to participate in clinical studies, especially when existing treatment protocols are not effective. In an interesting twist, LEAH is using a crowdfunding approach to try to develop its cure for cancer in dogs.

Companion animal ownership and expenditures have grown steady over the last 20 years, and in the US alone approximately $75 billion will be spent in 2019 on pets[12]. In addition to treating diseases in companion animals there is the potential for using CRISPR gene editing in trait and breeding development. Many pure breeds suffer from specific health issues, which could be addressed by combining with genetics from 'healthier' animals.

The Future

Since the discovery of CRISPR, the concept of applying genome editing techniques in areas outside of human healthcare has expanded tremendously. Where humans were the primary beneficiary of early drug studies in animals, it now appears that animals are beginning to benefit from the aggressive application of genome editing approaches being sought for human healthcare. Through broad and affordable access to this innovative technology, the field of animal health now has access to a cutting-edge technology that could revolutionise the thinking around animal health and wellbeing. We look forward to seeing the field take enormous strides in the coming years.

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