RECENT ADVANCES IN GENOME EDITING: OPPORTUNITIES IN LIVESTOCK PRODUCTION AND IN THE FIGHT AGAINST ANIMAL DISEASES

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With the World population estimated to increase to over 9 billion by 2050, Food and Agricultural Organization (FAO) estimates that the overall food production will have to increase by nearly 70% to ensure Global Food Security[1]. Ongoing changes in the climate, water shortage and continued reduction in the availability of agricultural land are major limitations in further expansion of agriculture. There is also additional pressure on the livestock sector to meet the growing demand for high-value animal proteins of milk, meat and eggs. The general strategy in the past for maximising the productivity of our livestock was mainly focused on improving the genetics of our animals. Humans have a very long history in shaping the genetic makeup of livestock to optimize production by traditional selective breeding. While this was largely successful as is evident from a number of examples we can see around us, it had been a painstakingly slow process of accumulating only incremental gains over a long period. Moreover, the levels of improvements by conventional selective breeding programmes that we have followed for the last several decades have almost plateaued, and further attempts to push the limits for more gains in productivity are unlikely to be economical and pragmatic in the time frame we need to achieve our goals in food security. In addition, genetic bottlenecks in selecting the desired alleles over undesired ones continue to be a limiting factor in conventional breeding.

Last 30 years have witnessed major technological advances in molecular biology that have significantly enhanced our capability in many areas of biosciences. Advances in sequencing technologies and reduction in the costs of genome sequencing have led to the determination of the complete genome sequences of a number of livestock species including cattle, pigs, sheep, goats and poultry. This has given us the unprecedented access to investigate the genetic determinants related to a number of traits such as those associated with productivity, growth rate, feed conversion ratio, disease resistance and even behaviour.

Development of transgenic technology has offered a more direct approach for genetic

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improvement. Although this had a more direct impact in the agricultural sector for improving productivity and disease resistance of a number of plant species, there are many examples of improved productivity in the livestock sector also by transgenic approaches. However, the recent advances in precision genome editing tools, and their relatively easy application, have reignited the opportunities for making revolutionary changes in livestock breeding for increasing productivity and disease resistance. The introduction of site-specific DNA double strand breaks by chimeric designer nucleases such as zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs) can induce gene disruptions or trigger homology-driven genome modifications with unprecedented ease.

However, these technologies have largely been superseded by the CRISPR (clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 (CRISPR-associated protein 9)system, which has emerged as a 'game changer' because of its simplicity and versatility in its application[2]. CRISPR/Cas9 is an RNA-based adaptive immune system used byarchaea and eubacteria to cleave foreign genetic elements such as viruses and plasmids. Currently, the most widely usedCRISPR/Cas9 system is a modified from *Streptococcus pyogenes* and consists of the guide RNA and the Cas9 endonuclease. These two components form a complex with the 20-nucleotide section of the guide sequence target the DNA by Watson-Crick base pairing, followed by cleavage by Cas9 tocreate double strand breaks (DSBs). Following the editor activity, DSB repair is carried out by the two cellular mechanisms, viz. imprecise non-homologous end joining (NHEJ) and precise homology-directed repair (HDR). The error prone NHEJ pathway, where the DSBs are processed by endogenous DNA repair machinery, results in mutations comprising of insertions/deletions (indels) at the site of the junction. The indelswithin the coding region cause frame shifts and premature stop codons resulting in the knock out of the gene function. When the functional knockout is not the only desired goal,HDR pathway can be exploited for more precise modifications of the genome including the introduction of replacement alleles, point mutations or modifications of codons.

The promise of genome editing in animals

The use of site-specific nucleases in genome engineering has been very successful in several plant species including maize, wheat, rice, soybean, tomato and potato. However, genetic engineering of livestock species had been more challenging and the technological advances did lag behind the development of plant-based tools. Nonetheless, the remarkable development of genome editing technologies in the last few years is set to revolutionise the field of livestock farming with huge benefits. Applications of these powerful genome editing tools in the livestock sector can deliver economic benefits through increased productivity and improved animal welfare and reduction of disease burden. The best example to show the power of gene editing in livestock is the modification of the POLLED gene in dairy cattle[3]. Horns were valuable in the survival of cattle in early domestication thousands of years ago. However in today's dairy stock, horns not only have any intrinsic value, but also pose risk to other livestock and humans. Comparison of the genome of horned and hornless cattle mapped the two point mutations on bovine genome that prevented the development of horns in certain breeds of cattle. While standard cross breeding programmes with hornless breeds can be used to establish the trait, it would take about 25 years of backcrossing and selection to recover all the dairy merits in such herds. Gene editing to introduce *hornless* trait saves about eight generations of backcrossing, without the need for screening for other alleles desirable to the industry. The successful use of genome editing to develop BLG-knockout cows[4]has helped to produce non-allergic milk, as BLG protein has been shown to be one of the major milk allergens. Similarly, one-step generation of triple gene-targeted pigs[5] demonstrate the simplicity, efficiency, and power of the CRISPR/Cas9 system for the modification of multiple genes in pigs for yielding results of high medical value.

There are reports that entire barnyards of genome-edited animals are rapidly filling the Research& Development pipeline across different nations. Recombinetics, one of the start-up biotechnology companies which first demonstrated the potential of genome editing for producing hornless dairy cattle, is working to produce beef cattle with larger muscles. Genome editing of the myostatin (MSTN) gene, a negative regulator of myogenesis and whose disruption typically results in increased skeletal muscle mass has already been shown in pigs, cows and sheep. Recent studies in China showed that the disruption of FGF5 in Cashmere goats using CRISPR/Cas9 increased secondary hair follicles and longer fibres that will significantly boost wool production. There are other companies trying to develop chickens that only produce female offspring for the egg laying industry, and beef cattle that only produce males for more efficient feed-to-meat conversion. These developments will certainly bring economic benefits, but will also have much smaller environmental footprint as such animals could yield more food with less pressure on the resources.

In addition to the direct attempts for increased productivity of economic traits such as milk, meat or egg, genome editing can bring in profound benefits in improving animal health through improved resistance to diseases. Recent studies at the Roslin Institute in the UK have led to the development of founder pigs with mutations in the RELA gene of the NF-κBsignalling cascade, a key regulator of the immune response that drives the symptoms of a major haemorrhagic highly fatal disease of pigs called African swine fever. Using genome editing approaches, researchers tweaked the genome of domestic pigs to achieve the exact warthog RELA sequence. This novel feat of animal genetic engineering, the precise and efficient substitution of an agronomic haplotype into a domestic species to render them genetically resistant to a major fatal infectious disease, opens unprecedented opportunities for advancing basic research in this important area. Further studies have shown that the modification of the CD163 gene creates the opportunity to develop production animals that are resistant to PRRS, a very costly viral disease to ever face the swine industry. Similarly in poultry there are attempts to produce bird resistant to lymphomas induced by avian leukosis viruses by genome editing of the specific receptor sequences. These approaches leading to increased genetic resistance to infectious diseases, will also be a valuable adjunct to the vaccination-based control strategies.

Transgenic animal platform for production of biopharmaceuticals

In the last five years, genome editing has been used to mediate the generation of more than 300 edited pigs, cattle, sheep and goats[6]. Some of these animals potentially serve asbioreactors for production of drugsor founder animals of genetic lines with enhanced productivity or as organ donors. The production of recombinant proteins for human therapeutics is a major source of innovation in the pharmaceutical industry today. Use of transgenic animals for the production of such proteins has been one of such innovations of the last decade, and there have been significant advances in using the transgenic animal platform for biopharmaceutical production[7]. Transgenic animals offer particularly attractive possibilities for the preparation of recombinant proteins at the fraction of price in bioreactors or cell culture systems. In the transgenic animal models used as expression systems for biopharmaceuticals, milk seems to be the mostprominent, proven and mature system for the productionof recombinant proteins, where the mammary gland is the main bioreactor path for recombinant protein production. Such expression systems can result in high level production of recombinant proteinssuch as the recombinant human albumin at 1–5 g/L of milk. The recombinant human antithrombin produced through transgenic goat's milk was approved for human use by FDA, further confirming the robustness and effectiveness of this platform for producing proteins for human therapeutics.

The potential of using transgenic hen eggs as live bioreactors for the production of human epidermal growth factor in the egg white of transgenic hens have also been demonstrated, by making use of an oviduct-specific minisynthetic promoter[8]. With each egg white capable of producing approximately 4 g of protein and a single hen able to lay up to 330 eggs per year, the potential of this system is enormous. Previous studies have also shown high levels of production (38 mg/L in the egg white)of recombinant human interferon in transgenic eggs. Another major potential of using transgenic animal platforms as bioreactorsof the future will be the production of monoclonal antibodies (MAbs), the current most valuable recombinant proteins in the market in biomedical and commercial interests. Recent success with the generation of 'humanized' cattle with entire bovine immunoglobulin loci were inactivated and replaced with the corresponding human immunoglobulin genes[9], opens up the possibility of production of large quantities of human antibodies from cows that could be administered directly in human patients. Similar approach of the chicken model for the production of human antibodies has also been recently reported[10]. Successful use of CRISPR-Cas9-based tools for genome editing in chickens[11], demonstrate the huge potential of using this biological system for improving production or fighting diseases.

Ethical considerations

While genome editing offers a potential set of responses to the challenge of developing and maintaininga sufficient supply of safe, nutritious food, there are a number of ethical issues that needs to be considered[12]. Given its imminence, and in contrast to the very considerable public debate that has surrounded genetically modified crops, comparatively less attention has been given to genetic livestock manipulation and its regulation. Much awareness has, however, been given to alternative methods of husbandry and the role of livestock of different kinds in meeting people's needs and desires for food. Many of the moral and societal issues are common to plants and animals, but they are not simply about securing adequate levels of consumption of safe, nutritious food. Lack of evidence of harm to human health of GMOs is cited as a reason to move to product regulation, based on substantial equivalence to existing products. Genome editing may play a potentially significant, though morally ambiguous, role in relation to sustainability, intensity, yield, human and animal welfare and quality. There are also the lines of thinking that gene editing is different from gene modification, as many of the changes introduced in the editing process do already exist in nature. Recent FDA approval[13] of the *AquAdvantage salmon*, the first genetically modified animal approved for human consumption is seen by many as a bold step which is likely to open up the gate for bringing further genome edited livestock to solve the major challenges of food security and poverty alleviation in many parts of the world.

With technologies of genome editing becoming more efficient with much less off-target effects, and the political landscape becoming less hostile to the concept, genome editing as a tool for improving livestock productivity and welfare through reduced infection burden is likely to emerge as a major game-changer in the coming years. The current crowded intellectual property landscape enveloping the genome editing technologies and the paths of legal challenges will resolve with time. We are only at the dawn of this wave of scientific advance—the world for livestock biotechnology is about to get very exciting.

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