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REVIEW

Gene engineering in swine for agriculture

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Abstract

Domestic pigs are the second most important source of meat world-wide, and the genetic improvement of economic traits, such as meat production, growth, and disease resistance, is a critical point for efficient production in pigs. Through conventional breeding and selection programs in pigs, which are painstakingly slow processes, some economic traits, such as growth and backfat, have been greatly improved over the past several decades. However, the improvement of many polygenetic traits is still very slow and challenging to be improved by conventional breeding strategies. The development of reproductive knowledge and a variety of techniques, including foreign gene transfer strategies, somatic cell nuclear transfer (SCNT) and particularly, recently developed nuclease-mediated genome editing tools, has provided efficient ways to produce genetically modified (GM) pigs for the dramatic improvement of economic traits. In this review, we briefly discuss the progress of genomic markers used in pig breeding program, trace the history of genetic engineering, mainly focusing on the progress of recently developed genome editing tools, and summarize the GM pigs which have been generated to aim at the agricultural purposes. We also discuss the specific challenges facing application of gene engineering in pig breeding, and future prospects.

Keywords: gene engineering, genome editing, pig, agricultural application

1. Introduction

Humans have a long history of investigating the genetic makeup of beneficial traits to optimize pig production to meet increasing global demands for high-quality pork, thereby contributing to human consumption habits and food security. In conventional selection and crossbreeding systems, to obtain genetic improvements in the pure lines that contribute to market production, multiple nucleus populations must be built and maintained with extensive selection, including phenotype recording, genetic evaluation, selection of parents, etc. The processes are painstakingly slow, however, through this strategy of genetic improvement, some economic traits, such as growth rate and backfat, have been improved rapidly (Chen *et al*. 2002).

Since the 1980s, genetic markers have been developed and applied in livestock improvement programs, which have shown great potential for overcoming the above limitations during selection. The earliest and most successful story is the Halothane gene genetic test in selection for meat quality (Fujii *et al*. 1991). Since then, and until the end of the last century, many scientists have attempted to

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identify genetic markers from microsatellites to single nucleotide polymorphisms (SNPs) that are associated with economically important traits *via* candidate gene approaches and quantitative trait loci (QTL) mapping. Some well-known economically important genes, including *ESR*, *RN*, *MC4R*, etc., have been identified (Van Eenennaam *et al*. 2014). The marker-assisted-selection (MAS) approach significantly improves the accuracy of breeding value estimations for monogenic traits. However, this is not the case for quantitative or polygenic traits with low heritability, such as traits of reproductive and meat quality.

At the beginning of this century, a dense set of genetic markers that are evenly spread throughout the genome was predicted to be able to overcome many limitations that were previously identified in traditional strategies and evaluate the genetic merit of individuals (Meuwissen *et al*. 2001). The 60K SNP panel for pigs was released in 2009, which allows for the genetic merit evaluation and selection in candidate breeding animals with more accuracy through genome-wide association analysis (GWAS), especially for polygenic traits (Ramos *et al*. 2009). By GWAS, the genomic markers controlling genetic variation in economically important pig phenotypes, including causative genes and QTLs, have been successfully identified (Ernst and Steibel 2013). However, regarding to the genome selection for pig breeding, the high cost of DNA isolation, genotyping and phenotypic data collection greatly limits its application. To reduce the cost without affecting the selection accuracy, trait-line-specific low-density panels were developed to genotype on dams and have been combined with high-density panels to genotype breeding males (Hickey *et al*. 2011, 2012). This strategy has been reported to be effective and has been applied in some large-scale pig breeding companies to select for specific traits (Van Eenennaam *et al*. 2014). In addition, N-ethyl-N-nitrosourea (ENU)-mediated artificial random mutagenesis in pigs has been reported very recently, which provided powerful tool to efficiently generate the reservoir of mutants at the genome levels and screen the mutants with desired alleles for agricultural and biomedical research (Hai *et al*. 2017). We summarized the timeline for the historical use of DNA markers in pig breeding programs, as well as the ENU-mediated mutagenesis at the genome levels in pigs (Fig. 1-A). No doubt, the conventional or 'artificial' selection program in pig is largely uncontroversial, however, due to the limitations we described above, the innovations in breeding strategies are expected to improve the pig production efficiently.

2. Techniques for genetic engineering

Over the past three decades, with the increasing ability to read and interpret pig genomes and with the development of modern biotechnologies, especially recently developed and optimized genome editing tools, desirable alleles can

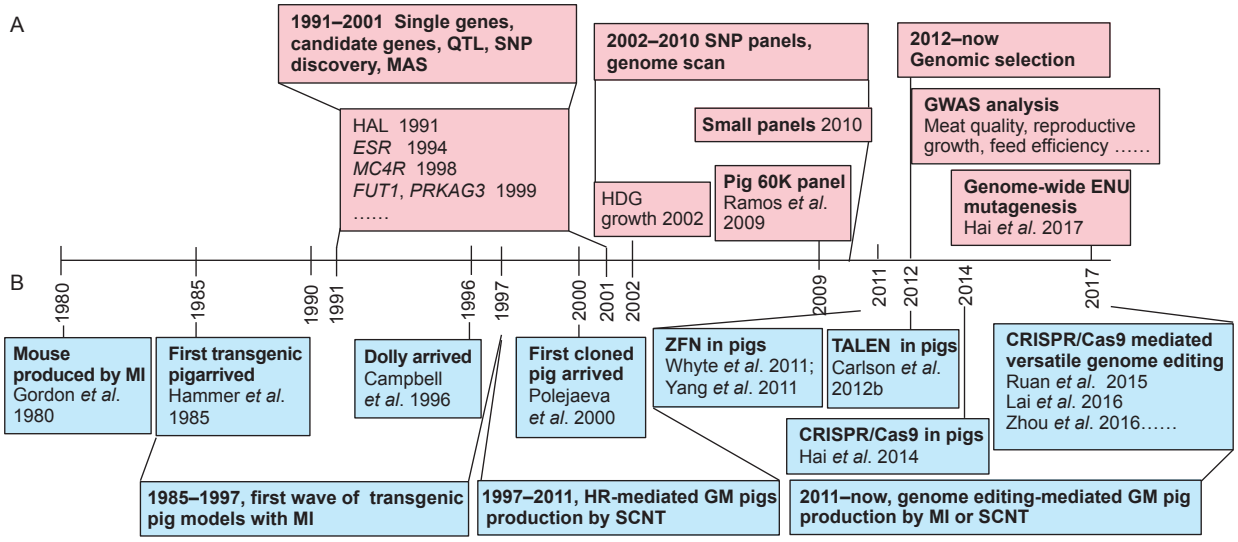

Fig. 1 A, timeline for progress of genomic markers used in conventional pig breeding programs. B, specific milestones of genetic engineering, genome editing tools and the generated genetically modified pigs over the past 35 years. QTL, quantitative trait loci; SNP, single nucleotide polymorphisms; MAS, marker assisted selection; HAL, halothane; ESR, estrogen receptor; MC4R, melanocortin 4 receptor; FUT1, fucosyltransferase 1; PRKAG3, protein kinase AMP-activated non-catalytic subunit gamma 3; GWA, genome-wide association study; ENU, N-ethyl-N-nitrosourea; MI, pronuclear microinjection; HR, homologous recombination; GM, genetically modification; SCNT, somatic cell nuclear transfer; ZFN, zinc finger nuclease; TALEN, transcription activator-like effector nuclease; CRISPR/Cas9, clustered regularly interspaced short palindromic repeat (CRISPR)/CRISPR-associated (Cas) protein 9 system.