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TRANSGENIC PIGS FOR BIOMEDICAL APPLICATIONS

Emoke Pall, Niculae Mihaela, Cenariu Mihai, Simona Ciupe, Groza Ioan

University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca, Romania,
pallemoke@gmail.com

Abstract
The purpose of this article is to review the most modern techniques used in obtaining transgenic pigs as important models in the study and understand of mechanisms involved in the pathogenesis of various human diseases. Genetically engineered animals have a significant role in biomedical research (Wheeler 2007, Robl et al., 2007, Vajta et al., 2012). Genetically modified swine are recognized as outstanding models for the study of various human diseases. These animals have high reproductive capacity and adequate precocity, short farrowing interval, parturition of multiple offspring (an average of 10–12 piglets per litter) (Wolf et al., 2000, Walters et al., 2012). Besides all these aspects the completed sequencing of the swine genome (Schook et al., 2005), made possible precise representations of human diseases based on propinquity of genes between the two species (Whyte, Prather 2011). Pigs are recognized as excellent models in a variety of areas, including nutrition, toxicology, dermatology, diabetes, cancer, atherosclerosis, cardiovascular disease, cutaneous pharmacology, ophthalmology, degenerative disease etc. (Lunney et al., 1999, Ishii et al., 2006, Herkenne et al., 2006, Dyson et al., 2006, Du et al., 2007, Matsunari, Nagashima 2009, Aigner et al., 2010, Noel et al., 2012, Prather et al., 2013). Due to the emergence of modern genetic engineering techniques, the genome of these animals can be modified to serve as a potential xenograft donor (Sachs, Galli 2009).

Key words: biomedical, pig, transgenic,xenograft

INTRODUCTION

Transgenic (TG) pigs generated using assisted reproductive techniques are favorable animal models often used in various fields of biomedical research (Douglas 1972, Furth et al., 1994, Phelps et al., 2003, Furth et al., 1994, Aigner et al., 2010, Hemann et al., 2012, Watanabe et al., 2012, Yeom et al., 2012, Giraldo et al., 2012, Jin Y.X et al., 2014).

The human and and pig have a large number of likeness in anatomy, physiology and pathophysiology (Larsen et al., 2004), thus genetically modified swine are recognized as excellent models for the study of various human diseases (Jin Y.X et al., 2014) and the development of new strategies for disease prevention and treatment.

This is due to the fact that these animals have high reproductive capacity and precocity, short farrowing interval, parturition of multiple offspring (10–12) combined with the availability of techniques for oocyte manipulation and artificial insemination (Wolf et al., 2000, Sachs et al., 2009, Walters et al., 2012).

The goal of transgenic technology refers to the insertion of foreign genes into livestock and its stable integration into the germ line (Wheeler et al., 2003) followed by the expression in tissues of the resulting individual.

Using such methods have already received numerous genetically modified strains of animals useful for xenotransplantation (Sachs et al., 2009).

Pronuclear microinjection

The first method to produce transgenic pigs, pronuclear microinjection, was originally initiate in mice (Brinster et al., 1981, Whyte, Prather 2011) and then extended in other species. This technique is based on the microinjection of DNA into the pronuclei of zygotes collected from a superovulated female, and then transferred to recipient animals by embryo transfer (Hammer et al., 1985, Whyte & Prather 2011).

This technique permits the addition of large transgene at a random location (Prather et al., 2013), but the major drawback is that only about 1% of injected eggs produce transgenic pigs (Niemann, 2004, Prather et al., 2008, Whyte & Prather 2011).

Sperm mediated gene transfer (SMGT)

SMGT is a technique to produce multitransgenic pigs with high efficiency based on the intrinsic ability of epididymal sperm cells to bind, internalize and integrate exogenous nucleic acid during fertilization (Lavitrano et al., 2005).

SMGT in the pig was carried out by collection of sperm, incubation of sperm with exogenous DNA, and artificial insemination of gilts with modified semen. A very important aspect refers to the careful selection of semen donor animals (Aigner et al., 2010). However, this method has limitations which refers limitations to the inability to prescreen embryos for transgene integration prior to embryo transfer (Whyte & Prather 2011)

Viral mediated transgenesis

Viral-mediated transgenesis are extremely efficient, with 80–100% of the animals being born transgenic after oocyte or embryo infection (Whitelaw et al., 2004), or somatic cell culture infection. The method requires the use of viruses to transduce cells with various transgenes. The viral strains that are often used are retroviruses belong to the family *Retroviridae.*

These viruses insert a DNA copy of their genetic material, produced from RNA as a template, into the host cell DNA following infection (Wheeler &Walters 2001).

The main advantage of retroviral-mediated gene transfer into animals is the technical ease of presenting a virus to embryos at various developmental stages (Wheeler &Walters 2001), but the major disadvantage of this method refers to the risks associated with multiple integration including oncogene activation, insertional mutagenesis and silencing of lentiviral sequences (Hofmann et al., 2006) and high frequency of mosaicism in obtained animals.

Somatic Cell Nuclear Transfer (SCNT)

Somatic cloning is emerging as a new biotechnology, has been established using blastomeres from early stage embryos as donor cells (Niemann & Lucas-Hahn 2012). SCNT works better in pigs than in other large animals (Lagutina et al., 2007). Embryonic stem cells (ESCs) and embryonic germ cells (Shim et al., 1997, Piedrahita et al., 1998) are important sources of cells for the production of transgenic animals. This transfer method involves injection of embryonic cells into expanded blastocysts to produce chimeric embryos composed of two or more distinct cell lines (Robertson et al., 1986).

Transgenesis by SCNT involves the following steps: (1) genetic modification and selection of donor cells in culture; (2) recovery and enucleation of in vivo or in vitro matured oocytes (metaphase II); (3) nuclear transfer by electrofusion and activation; (4) in vitro culture of the reconstructed embryos; and (5) embryo transfer to synchronized recipients (Aigner et al., 2010).

ESCs has many advantages for transgenesis (Robertson 1991); these advantages include: ESCs can be isolated from a single cell, the capacity of self-renewal without senescence, transformed ES cells can be screened and selected using reporter genes, ESCs cells can be transformed *in vitro* with foreign DNA, these cells can be expressed in tissues and organs.

The ability of the isolated ESCs cells to participate in embryogenesis of porcine chimeras was tested in different stage of embryos development (morula, blastocyst and expanded blastocyst stage embryos injected with embryonic stem cells). No differences were observed between embryonic stages. The major limitations of this method are represented by the lack of embryonic stem cell (ESCs) technologies.

Adult cells can be used for transfer but they have a limited lifespan, thus restricting the time the cells can be cultured *in vitro* for genetic manipulation (Sachs et al., 2009).

The production of transgenic organisms represented a major technical advance in many research areas. The numbers of animals obtained by these methods have grown exponentially in recent years, also have been developed standardized protocols globally accessible to more researchers (Whyte & Prather 2011).

The development of genetically modified pigs in various medical purposes has a significant impact for the scientific community and improving the development of treatments and new therapies for human diseases. Currently, genetically modified pig models are used for the analysis of gene function in a variety of human diseases, the development of new therapeutic strategies, and the production of biopharmaceuticals (Walters et al., 2012).

Complete pig genome sequencing and the modern techniques, now offer very useful tools for veterinary and human medicine. The research results conducted on these animals can be extrapolated in various medical fields.

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