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RESEARCH ARTICLE

Identification of the miniature pig inbred line by skin allograft

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Abstract

Skin grafting has been used as one of the most reliable tests to determine the genetic stability of laboratory animal such as mice and rats inbred line, but no identification of swine inbred lines by skin grafting has been reported. At present, Wuzhishan miniature pig (WZSP) inbred line has acquired the F_{24} individuals in China. In order to verify whether WZSP inbred line had been cultivated successfully, allogeneic skin grafts and related research were performed on F_{20} individuals of WZSP inbreeding population, compared with a control group of autologous transplantation. We observed the transplant recipients' wounds, detected peripheral blood-related indicators interleukin-2, 4 and 10, CD4⁺ and CD8⁺ lymphocytes, and conducted hematoxylin-eosin (HE) and Masson's staining of skin to judge whether the immune rejection reactions occurred within 28 days after transplantation. Chr. 7 genomic heterozygosity of 48 WZSP individuals from F_{20} to F_{22} was analyzed by high-density single nucleotide polymorphism (SNP) chips (60 000 SNPs). The result showed that there were no significant differences in graft skin, the plasma interleukin-2, 4, 10, CD4⁺ and CD8⁺, HE and Masson's staining results between the allograft and autograft groups, and no immune rejection occurred on the allograft group. We found that 11 genes in Chr. 7 of major histocompatibility complex (MHC) I and MHC II were homozygous which confirmed that immune antibody of the allograft and autograft groups were highly identical and also provided a theoretical basis to no immune rejection occurred on the allograft in the inbred WZSP. The result proved that the WZSP inbred line had been cultivated successfully for the first time in the world. The test methods also provide a scientific basis for the identification of swine and mammal inbred lines.

Keywords: Wuzhishan miniature pigs, inbred line, skin allograft, immune rejection

1. Introduction

^{*} These authors contributed equally to this study.

Skin grafting has been used as one of the most reliable tests to determine the genetic stability of laboratory animal mice and rats inbred line. Therefore, appraisal standard of genetic test on laboratory animals inbred lines including mice and rats by skin grafting has been promulgated internationally (Bailey *et al.* 1960; Benson *et al.* 1971; Chen *et al.* 1981; GB/T 14927.2-2001 2001). A series studies on skin allograft grafting and related mechanism have been done (Wang

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et al. 2001; Qiu *et al.* 2006; Zhou *et al.* 2006; Horner *et al.* 2009; Jiang *et al.* 2009; Dai *et al.* 2012; Kwak *et al.* 2013), however, there are no related studies on swine inbred lines or identification of swine inbred lines by skin grafting have been reported.

The inbred Wuzhishan miniature pig (WZSP) was developed by the Institute of Animal Sciences, Chinese Academy of Agricultural Sciences. The founders of the inbred line were one boar and one sow of full-sib in Hainan Province, China. The two animals were transported to Beijing in 1989 to initiating the development of the line. During 25 years, a mixed mating scheme of full-sib and parent-offspring improves the level of nutrition and synchronous estrus, the comprehensive measures such as outdoor sports and cage rearing are carried out, the inbred lines overcome "3 high 1 low" bad influence (high mummies and weakling piglet rate, high mortality, and low survival rate), and piglet survival rate is raised gradually from less than 20% at F, generation to about 90% at F₂₀ generation. The inbreeding coefficient reached 0.994 in F_{24} generations (2013). Compared with the original WZSP breed and also other varieties of minipigs, the inbred WZSP lines have gentle temperament and present uniform body colour and size. Its genetic stability has also been detected by a series researches on molecular

genetics (Li *et al.* 2009a, b; Feng 2011; Cheng *et al.* 2012; Fang *et al.* 2012). The technology systems of establishing the inbred line and associated breed standard were authorized by two invention patents (ZL.02149030.9 and ZL.2005.10008994.2). A large number of applications have shown that the inbred line is a new animal model for human due to the same size of organ and a higher homology genes of cardiovascular disease and drug metabolism disease genes and so on with human (Zhou *et al.* 2006; Feng *et al.* 2011, 2013). In order to verify whether WZSP inbred line had been cultivated successfully, autologous and allogeneic skin transplantations and related research were performed on F_{ao} individuals of WZSP inbreeding population.

2. Results

2.1. Wound observation and related research

The results of allogeneic and autologous transplantation groups are showed in Fig. 1.

During the initial 14 days after skin transplantation, the transplanted skins in both groups became red, indicating that the skins were able to establish a blood supply, hence, new blood vessels were developed, and almost 100% of



Fig. 1 Skin statuses on 7–28 days after transplantation.

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