資料１－３

ブタのゲノム編集

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養豚業におけるウイルス感染症の制御

PRRSウイルス受容体を欠失したブタの作出に成功

PLoS Pathog. 2017 Feb 23;13(2):e1006206. doi: 10.1371/journal.ppat.1006206. eCollection 2017 Feb.

Precision engineering for PRRSV resistance in pigs: Macrophages from genome edited pigs lacking CD163 SRCR5 domain are fully resistant to both PRRSV genotypes while maintaining biological function.

Burkard C[1], Lillico SG[1], Reid E[2], Jackson B[2], Mileham AJ[3], Ait-Ali T[1], Whitelaw CB[1], Archibald AL[1].

Abstract

Porcine Reproductive and Respiratory Syndrome (PRRS) is a panzootic infectious disease of pigs, causing major economic losses to the world-wide pig industry. PRRS manifests differently in pigs of all ages but primarily causes late-term abortions and stillbirths in sows and respiratory disease in piglets. The causative agent of the disease is the positive-strand RNA PRRS virus (PRRSV). PRRSV has a narrow host cell tropism, limited to cells of the monocyte/macrophage lineage. CD163 has been described as a fusion receptor for PRRSV, whereby the scavenger receptor cysteine-rich domain 5 (SRCR5) region was shown to be an interaction site for the virus in vitro. CD163 is expressed at high levels on the surface of macrophages, particularly in the respiratory system. Here we describe the application of CRISPR/Cas9 to pig zygotes, resulting in the generation of pigs with a deletion of Exon 7 of the CD163 gene, encoding SRCR5. Deletion of SRCR5 showed no adverse effects in pigs maintained under standard husbandry conditions with normal growth rates and complete blood counts observed. Pulmonary alveolar macrophages (PAMs) and peripheral blood monocytes (PBMCs) were isolated from the animals and assessed in vitro. Both PAMs and macrophages obtained from PBMCs by CSF1 stimulation (PMMs) show the characteristic differentiation and cell surface marker expression of macrophages of the respective origin. Expression and correct folding of the SRCR5 deletion CD163 on the surface of macrophages and biological activity of the protein as hemoglobin-haptoglobin scavenger was confirmed. Challenge of both PAMs and PMMs with PRRSV genotype 1, subtypes 1, 2, and 3 and PMMs with PRRSV genotype 2 showed complete resistance to viral infections assessed by replication. Confocal microscopy revealed the absence of replication structures in the SRCR5 CD163 deletion macrophages, indicating an inhibition of infection prior to gene expression, i.e. at entry/fusion or unpacking stages.

感染試験を実施！

PRRSウイルス受容体を欠失したブタは、同ウイルスの感染に抵抗性有

J Virol. 2018 Jul 31;92(16). pii: e00415-18. doi: 10.1128/JVI.00415-18. Print 2018 Aug 15.

Pigs Lacking the Scavenger Receptor Cysteine-Rich Domain 5 of CD163 Are Resistant to Porcine Reproductive and Respiratory Syndrome Virus 1 Infection.

Burkard C[1], Opriessnig T[2,3], Mileham AJ[4], Stadejek T[5], Ait-Ali T[2], Lillico SG[2], Whitelaw CBA[2], Archibald AL[2].

Abstract

Porcine reproductive and respiratory syndrome virus (PRRSV) has a narrow host cell tropism, limited to cells of the monocyte/macrophage lineage. CD163 protein is expressed at high levels on the surface of specific macrophage types, and a soluble form is circulating in blood. CD163 has been described as a fusion receptor for PRRSV, with the scavenger receptor cysteine-rich domain 5 (SRCR5) region having been shown to be the interaction site for the virus. As reported previously, we have generated pigs in which exon 7 of the CD163 gene has been deleted using CRISPR/Cas9 editing in pig zygotes. These pigs express CD163 protein lacking SRCR5 (ΔSRCR5 CD163) and show no adverse effects when maintained under standard husbandry conditions. Not only was ΔSRCR5 CD163 detected on the surface of macrophage subsets, but the secreted, soluble protein can also be detected in the serum of the edited pigs, as shown here by a porcine soluble CD163-specific enzyme-linked immunosorbent assay (ELISA). Previous results showed that primary macrophage cells from ΔSRCR5 CD163 animals are resistant to PRRSV-1 subtype 1, 2, and 3 as well as PRRSV-2 infection *in vitro* Here, ΔSRCR5 pigs were challenged with a highly virulent PRRSV-1 subtype 2 strain. In contrast to the wild-type control group, ΔSRCR5 pigs showed no signs of infection and no viremia or antibody response indicative of a productive infection. Histopathological analysis of lung and lymph node tissue showed no presence of virus-replicating cells in either tissue. This shows that ΔSRCR5 pigs are fully resistant to infection by the virus.**IMPORTANCE** Porcine reproductive and respiratory syndrome (PRRS) virus (PRRSV) is the etiological agent of PRRS, causing late-term abortions, stillbirths, and respiratory disease in pigs, incurring major economic losses to the worldwide pig industry. The virus is highly mutagenic and can be divided into two species, PRRSV-1 and PRRSV-2, each containing several subtypes. Current control strategies mainly involve biosecurity measures, depopulation, and vaccination. Vaccines are at best only partially protective against infection with heterologous subtypes and sublineages, and modified live vaccines have frequently been reported to revert to virulence. Here, we demonstrate that a genetic-control approach results in complete resistance to PRRSV infection *in vivo* CD163 is edited so as to remove the viral interaction domain while maintaining protein expression and biological function, averting any potential adverse effect associated with protein knockout. This research demonstrates a genetic-control approach with potential benefits in animal welfare as well as to the pork industry.

中国を中心に高致死率の高病原性PRRSが発生

PRRSウイルス受容体を欠失したブタは、高病原性PRRSの感染に抵抗性有

Int J Biol Sci. 2019 Jan 1;15(2):481–492. doi: 10.7150/ijbs.25862

Generation of Pigs Resistant to Highly Pathogenic-Porcine Reproductive and Respiratory Syndrome Virus through Gene Editing of CD163

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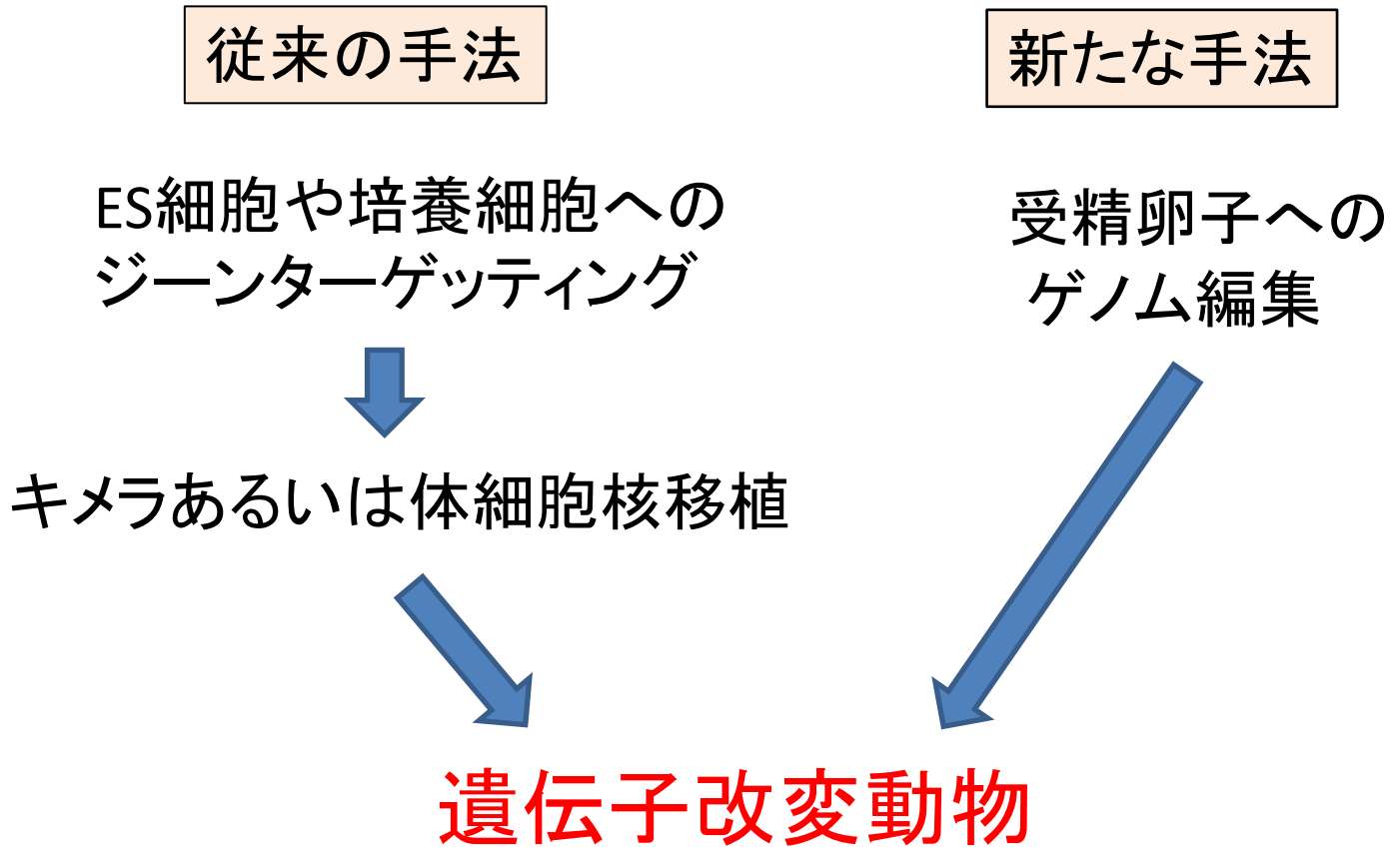
Abstract

Porcine reproductive and respiratory syndrome (PRRS) is a highly contagious disease and the most economically important disease of the swine industry worldwide. Highly pathogenic-PRRS virus (HP-PRRSV) is a variant of PRRSV, which caused high morbidity and mortality. Scavenger receptor CD163, which contains nine scavenger receptor cysteine-rich (SRCR) domains, is a key entry mediator for PRRSV. A previous study demonstrated that SRCR domain 5 (SRCR5), encoded by exon 7, was essential for PRRSV infection in vitro. Here, we substituted exon 7 of porcine CD163 with the corresponding exon of human CD163-like 1 (hCD163L1) using a CRISPR/Cas9 system combined with a donor vector. In CD163Mut/Mut pigs, modifying CD163 gene had no adverse effects on hemoglobin-haptoglobin (Hb-Hp) complex clearance or erythroblast growth. In vitro infection experiments showed that the CD163 mutant strongly inhibited HP-PRRSV replication by inhibiting virus uncoating and genome release. Compared to wild-type (WT) pigs in vivo, HP-PRRSV-infected CD163Mut/Mut pigs showed a substantially decreased viral load in blood and relief from PRRSV-induced fever. While all WT pigs were dead, there of four CD163Mut/Mut pigs survived and recovered at the termination of the experiment. Our data demonstrated that modifying CD163 remarkably inhibited PRRSV replication and protected pigs from HP-PRRSV infection, thus establishing a good foundation for breeding PRRSV-resistant pigs via gene editing technology.

ジーンターゲッティングによる哺乳動物の遺伝子改変技術



ゲノム編集によるジーンターゲッティング

DNA二本鎖切断後の修復機構

相同組み換え

・相同染色体による正確なDNA修復が生じるが、発生頻度は低い。
・姉妹染色分体形成時に生じる。

非相同末端結合

・細胞周期に依存しないため、発生頻度は高い。
・DNA修復時のDNAの欠失や挿入によるエラーが生じやすい。

マイクロホモロジー媒介末端結合

・頻度は低く、エラーが生じやすい。

従来法

ゲノム編集

ジーンターゲッティング

受精卵へのゲノム編集における問題点

1，オフターゲッティング

従来のジーンターゲッティングでは、ベクターは導入されるものの、ポジティブ・ネガティブセレクションにより標的外での変異は生じなかった。しかし、ゲノム編集ではPAM配列に依存した標的外での変異が生じる恐れがある。

2．モザイクの発生

・ゲノム編集では、標的とするDNA配列の切断後、主に、非相同末端結合による修復過程におけるDNAの欠失や挿入などのランダムなエラーを利用する。そのため、標的内の変異は一様ではない。

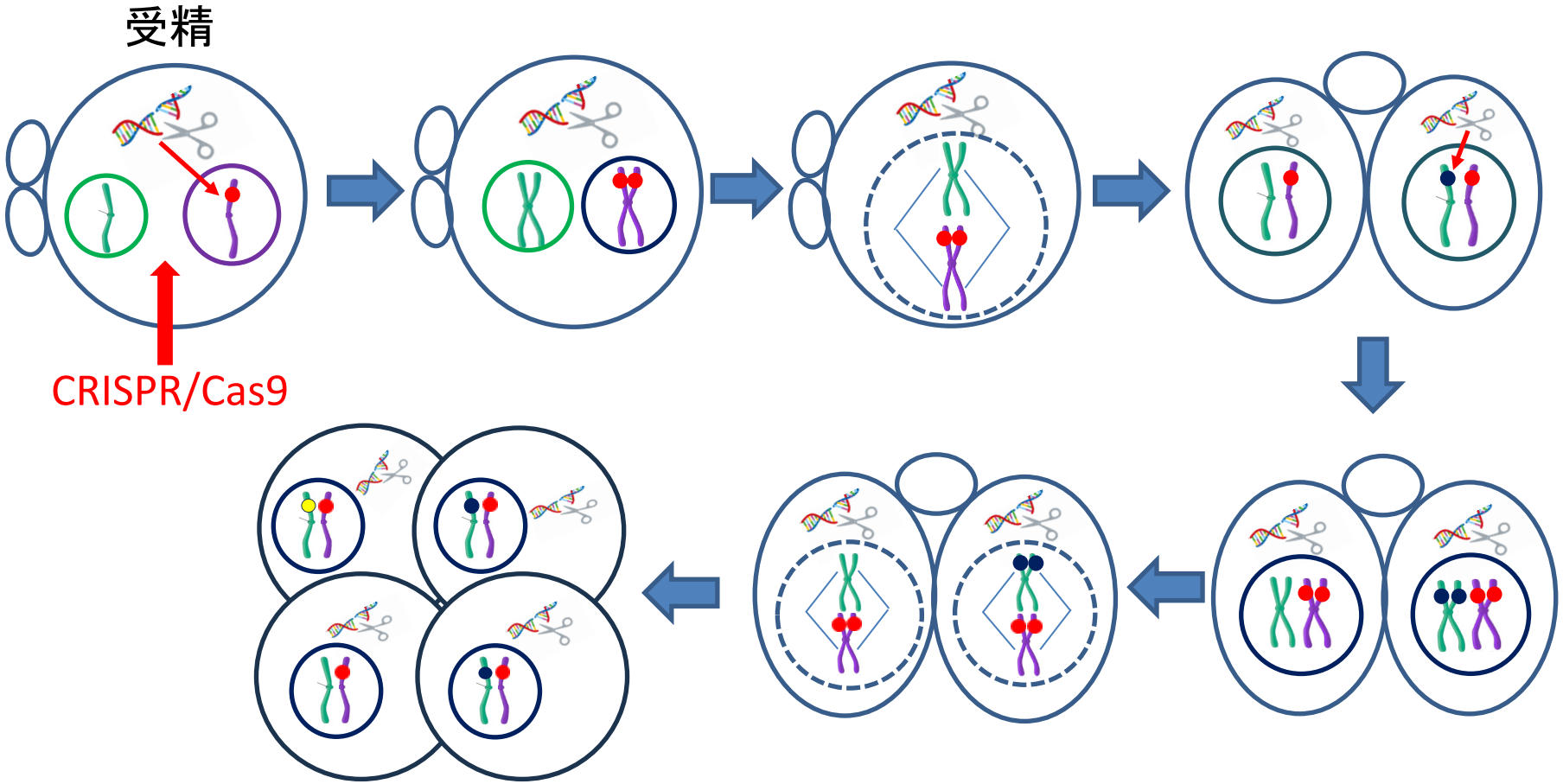
・受精卵への導入後、２～４細胞期においてもCRISPR/CASの機能が維持される。そのため、変異が継続して誘導され、種々の変異が細胞間で生じる。

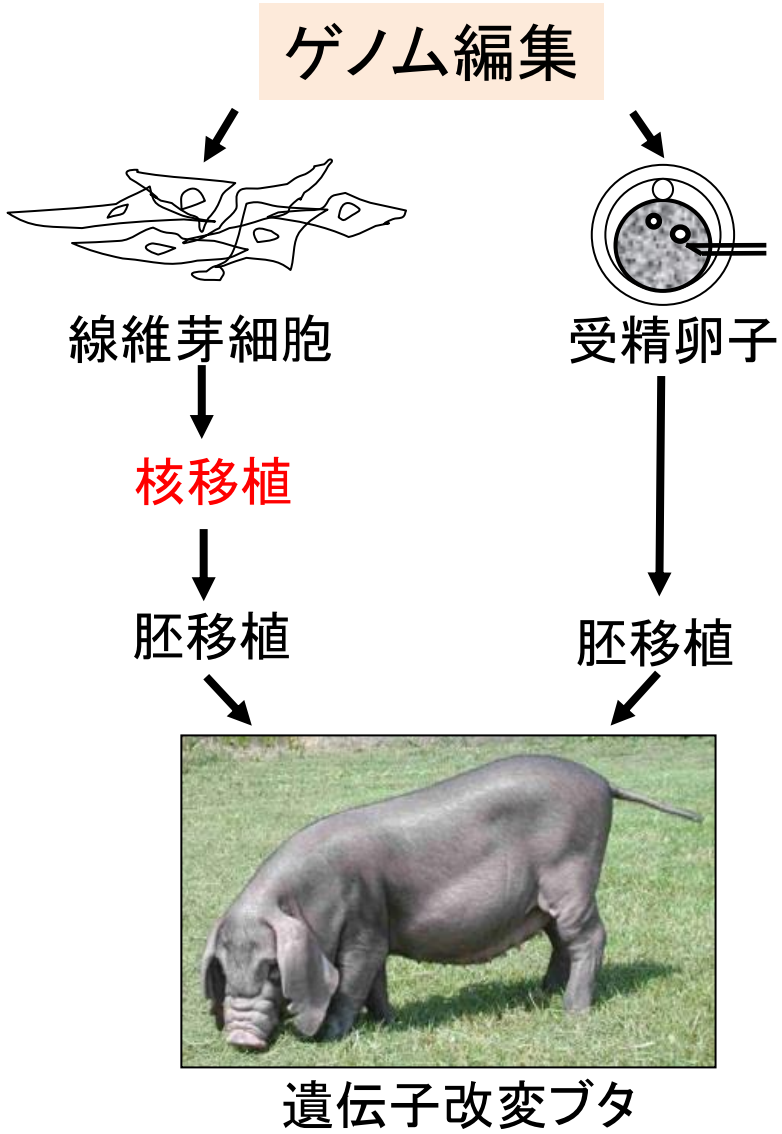
3．フレームシフトに伴う定形外翻訳

修復過程でのDNAの挿入や欠失に伴い、フレームシフトが生じて定形外翻訳が生じる恐れがある。

受精卵子へのゲノム編集でモザイクが生じる過程

1細胞期の細胞質に注入されたCRISPR/Cas9は、ブタでは4細胞期においても機能が維持されるため、種々の変異を伴うモザイクが発生する。なお、非相同末端結合は、姉妹染色分体の存在は必要なく、全ての細胞周期で生じる。





受精卵子へのゲノム編集

受精卵子へのゲノム編集は容易だが、モザイクは避けられない。変異を固定するには、後代の育種選抜が欠かせない。しかし、ブタの育種選抜には、多大な費用と時間を要し、必ずしも現実的ではない。

培養細胞へのゲノム編集

培養細胞へゲノム編集後、細胞を選抜して特定の変異細胞を得る。この細胞を核移植に用いることにより、モザイクを回避することができる。しかし、クローン特有のエピジェネティックな異常が生じる恐れがある。

ゲノム編集による遺伝子改変ブタの作出法

食用豚のライフサイクル

種付け（交配 または人工授精）

妊娠期間　114日

↓

哺育

約1か月で離乳

↓

育成・肥育

出荷 ← 肉用　6か月齢（体重110～115kg）

繁殖用　8か月齢 ← 種付け

（交配 または 人工授精）

↓

3～4回 分娩
約3年飼育

ブタの育種選抜では、1年間で1世代の更新となる。