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Disrupting the male germ line to find infertility and contraception targets

Décomposer la lignée germinale masculine afin d'identifier les cibles de l'infertilité et de la contraception

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Abstract

Genetically-manipulated mouse models have become indispensible for broadening our understanding of genes and pathways related to male germ cell development. Until suitable *in vitro* systems for studying spermatogenesis are perfected, *in vivo* models will remain the gold standard for inquiry into testicular function. Here, we discuss exciting advances that are allowing researchers faster, easier, and more customizable access to their mouse models of interest. Specifically, the trans-NIH Knockout Mouse Project (KOMP) is working to generate knockout mouse models of every gene in the mouse genome. The related Knockout Mouse Phenotyping Program (KOMP2) is performing systematic phenotypic analysis of this genome-wide collection of knockout mice, including fertility screening. Together, these programs will not only uncover new genes involved in male germ cell development but also provide the research community with the mouse models necessary for further investigations. In addition to KOMP/KOMP2, another promising development in the field of mouse models is the advent of CRISPR (clustered regularly interspaced short palindromic repeat)-Cas technology. Utilizing 20 nucleotide guide sequences, CRISPR/Cas has the potential to introduce sequence-specific insertions, deletions, and point mutations to produce null, conditional, activated, or reporter-tagged alleles. CRISPR/Cas can also successfully target multiple genes in a single experimental step, forgoing the multiple generations of breeding traditionally required to produce mouse models with deletions, insertions, or mutations in multiple genes. In addition, CRISPR/Cas can be used to create mouse models carrying variants identical to those identified in infertile human patients, providing the opportunity to explore the effects of such mutations in an *in vivo* system. Both the KOMP/KOMP2 projects and the

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CRISPR/Cas system provide powerful, accessible genetic approaches to the study of male germ cell development in the mouse. A more complete understanding of male germ cell biology is critical for the identification of novel targets for potential non-hormonal contraceptive intervention.

Keywords: Contraception; CRISPR-Cas; Germ cell; Infertility; KOMP; KOMP2; Spermatogenesis

Résumé

Les modèles de souris génétiquement manipulées sont devenus indispensables à une meilleure compréhension des gènes et voies de signalisation impliqués dans le développement des cellules germinales masculines. Tant que les systèmes d'études *in vitro* de la spermatogenèse ne se seront pas perfectionnés, les modèles *in vivo* resteront l'étalon d'or des investigations en matière de fonction testiculaire. Dans cet article, nous exposons les avancées stimulantes qui permettent aux chercheurs d'avoir accès de façon plus rapide, aisée et paramétrable aux modèles de souris d'intérêt. Plus spécifiquement, le projet *trans-NIH Knockout Mouse Project* (KOMP) a pour but de créer des modèles de souris invalidés pour chaque gène du génome de la souris. Le programme apparenté *Knockout Mouse Phenotyping Program* (KOMP2) réalise une analyse phénotypique systématique de cette collection de souris invalidées sur le génome, incluant une analyse de la fertilité. Ensemble, ces programmes permettront non seulement de découvrir de nouveaux gènes impliqués dans le développement des cellules germinales masculines, mais aussi de fournir à la communauté scientifique des modèles de souris nécessaires à des investigations ultérieures. Au-delà du programme KOMP/KOMP2, un autre champ de développement prometteur dans le domaine des modèles de souris correspond au projet CRISPR (*clustered regularly interspaced short palindromic repeat*)-Cas technology. En utilisant 20 séquences nucléotidiques guides, CRISPR/Cas a le potentiel d'insérer une séquence spécifique, ou à l'inverse d'introduire une délétion ou une mutation ponctuelle pour produire des allèles d'expression nulle, conditionnée, activés ou des allèles reporter marqués. La technologie CRISPR/Cas peut également cibler de multiples gènes en une seule étape expérimentale, évitant ainsi les multiples générations d'élevage traditionnellement requis pour produire ces modèles de délétions, insertions, ou mutations sur de multiple gènes. De plus, la technique CRISPR/Cas peut être utilisée pour créer des modèles de souris portant des variants identiques à ceux identifiés chez les hommes infertiles, offrant ainsi une opportunité d'explorer les effets de telles mutations sur un système *in vivo*. Les projets KOMP/KOMP2 et la technique CRISPR/Cas offrent ainsi une approche génétique puissante et accessible à l'étude de la lignée germinale masculine de la souris. Une compréhension plus approfondie de la biologie des lignées germinales masculines est essentielle à l'identification de nouvelles cibles contraceptives non hormonales efficaces.

Mots clés : Contraception ; CRISPR-Cas ; Cellule germinale ; Infertilité ; KOMP ; KOMP2 ; Spermatogenèse

Scientists interested in the development and function of the male germ line face a great number of challenges. Since spermatogenesis cannot be fully studied *in vitro*, animal models – in particular, genetically-manipulated mice – are a critical tool in our field. However, establishing animal models with disrupted germ cell function is inherently difficult since the very genes we are interested in disrupting are often critical for fertility. Many genes of interest in germ cell biology are critical for the development and/or function of other organ systems and result in embryonic or early postnatal lethality when ubiquitously knocked out/silenced. In addition, establishment of a "traditional" transgenic knockout mouse strain can be a time-consuming and expensive process. Even simple mouse husbandry chores such as breeding and genotyping can become extremely time-consuming, particularly when it becomes necessary to breed for double- or triple-knockout mice to elucidate the functions of a gene family or to understand a particular pathway. Here, we discuss the KOMP/KOMP2 initiatives and CRISPR-Cas tools and the opportunities they present for researchers interested in quickly establishing new mouse models in which to study the complexities of male germ cell biology.

1. KOMP and KOMP2

The Knockout Mouse Project (KOMP) was launched in 2006 as a trans-NIH initiative involving cooperative efforts between the Children's Hospital Oakland Research Institute (CHORI), the School of Veterinary Medicine at University of California, Davis (UC Davis), the Wellcome Trust Sanger Institute (WTSI), and Velocigene, a division of Regeneron Pharmaceuticals, Inc. [1]. The KOMP initiative seeks to generate a resource of embryonic stem (ES) cells containing reporter-marked null mutations of every gene in the mouse genome. The two KOMP mutagenesis teams are each utilizing a unique construct design to achieve this goal (for more information, see the KOMP website at: www.komp.org/faq.php#faq04a) [2]. The CSD consortium (CHORI, UC Davis, and WTSI) utilizes a trapping cassette (including a splice acceptor and lacZ reporter) flanked by Flp recombinase recognition sites ("FRT") which, when inserted into an intron upstream of a critical exon, undergoes splicing to result in a lacZ-tagged insertion allele predicted to be null due to truncation of the endogenous transcript (Fig. 1). The CSD targeting design also includes Cre recombinase target sites ("loxP") flanking the critical exons. Crossing the lacZ-tagged insertion allele to a Cre recombinase-expressing mouse strain deletes the "floxed" critical exons and results in a lacZ-tagged null allele. Inclusion of both the FRT and loxP sites into this design allows for the conversion of the LacZ-tagged null allele to an untagged, conditional-ready allele by crossing to a mouse strain expressing Flp recombinase. This post-Flp conditional-ready allele can then be converted to an untagged conditional null allele by crossing to a mouse