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Transgenic mouse models to study Gpr54/kisspeptin physiology

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ABSTRACT

Four transgenic mouse lines have been generated with mutations in the *Gpr54* gene and two lines with mutations in the *Kiss1* gene. In general, the phenotypes of all these mutant mice are very similar and provide evidence that these molecules constitute an authentic receptor/ligand pair with no obvious redundancy or overlap with other signaling pathways. The mutant mice all fail to undergo pubertal maturation and show poor development of the gonads and infertility with low sex steroid and gonadotrophic hormone levels (hypogonadotrophic hypogonadism). Spermatogenesis and ovulation are severely impaired and mutant females do not show estrous cycling. The gonads and the anterior pituitary retain functional responses to hormonal stimulation however, consistent with the primary defect being a failure to secrete gonadotrophin releasing hormone (GnRH) from the hypothalamus. Slight differences between the phenotype of some of the mutant lines may reflect the type of mutation carried by each line. These mutant mice are being used to interrogate the function of *Gpr54* and *Kiss1* in key aspects of mammalian reproduction *in vivo* including the role of these proteins in the generation of the pre-ovulatory luteinizing hormone (LH) surge and aspects of sexual behavior. They provide a useful resource to further understand the hypothalamic regulation of mammalian reproduction, its integration with the pituitary–gonadal axis and to study the potential function of *Gpr54* and *Kiss1* in peripheral tissues.

1. Introduction

Hormonal feedback loops within the hypothalamic–pituitary–gonadal axis control mammalian fertility. A key event at puberty is the initiation of pulsatile gonadotrophin releasing hormone (GnRH) secretion from the hypothalamus which stimulates gonadotrophin release from the anterior pituitary. The gonadotrophins, luteinizing hormone (LH) and follicle-stimulating hormone (FSH) act on the gonads to stimulate sex steroid production and gametogenesis. Two key proteins have been identified that are required for the pubertal activation of GnRH secretion and regulation of mammalian fertility. These proteins are the G-protein coupled receptor GPR54 and peptide ligands (kisspeptins) for this receptor encoded by the *Kiss1* gene. Transgenic mice with mutations in *Gpr54* or *Kiss1* have played an important part in establishing the importance of these genes in regulating fertility and unravelling how these molecules interact with other parts of the reproductive axis *in vivo*. They allow a detailed investigation into the anatomical, developmental and molecular consequences of loss of GPR54/kisspeptin signaling.

2. *Gpr54* and *Kiss1* transgenic lines

Four *Gpr54* and two *Kiss1* transgenic lines have been generated by manipulation of mouse embryonic stem cells. Three of the *Gpr54* lines were generated by gene targetting and carry deletions of different lengths of the *Gpr54* coding sequence (Fig. 1). The fourth line was generated by retroviral insertion at

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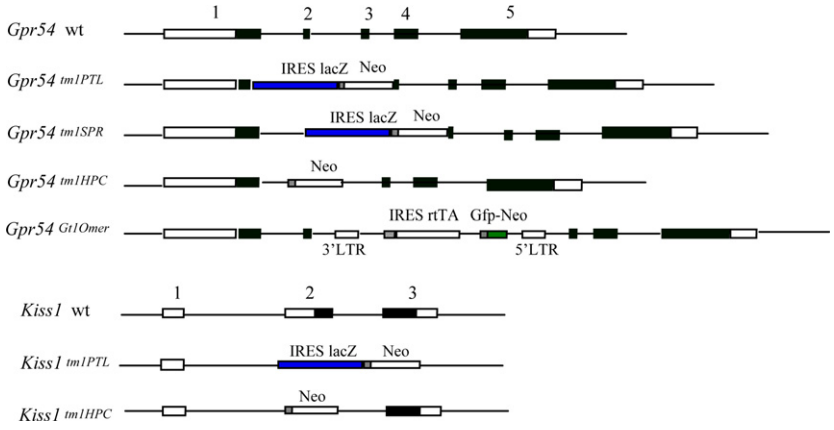

Fig. 1 – Genomic arrangement of *Gpr54* and *Kiss1* targetted loci. Coding regions in exons are shown in black. IRES, internal ribosome entry sequence; promoters are cross-hatched; neo, neomycin resistance gene; gfp, green fluorescence protein gene; SA, splice acceptor sequence; rtTA, reverse tetracycline-responsive transactivator protein, LTR, retroviral long terminal repeat; ^{tm}, targetted mutation; ^{Gt}, gene trap mutation. ^{PTL}, Paradigm Therapeutics Ltd; ^{SPR}, Schering Plough Research; ^{HPC}, Harvard Partners Center; ^{Omer}, Omeros Corporation.

the *Gpr54* locus (Fig. 1). The nomenclature for these transgenic lines is based on the guidelines established by the International Committee on Standardized Genetic Nomenclature for mice (http://www.informatics.jax.org/mgihome/nomen/index.shtml).

3. Mutations

The precise mutation present in each of these transgenic lines is important in understanding the possible differences in phenotype as well as the utility of each line for specific experimental studies (Table 1). The *Gpr54*^{tm1PTL} line (generated by Paradigm Therapeutics and associated with the Colledge laboratory) has a 702-bp deletion of the *Gpr54* gene consisting of the final 92 bp of exon 1, the whole of intron 1 (509 bp) and the first 101 bp of exon 2 [26]. Confirmation that this targetting event has produced a null allele was shown by RT-PCR which failed to detect transcripts downstream of the insertion. The *Gpr54*^{tm1SPR} line (generated by Schering Plough) has a 52-bp deletion within exon 2 and a null mutation in this line was confirmed by northern blot analysis using brain tissue [10]. The *Gpr54*^{tm1HPC} line (generated by the Harvard Partners Centre and associated with the Seminara laboratory) has complete

Table 1 – Summary of transgenic lines

Mouse line	Type of mutation	Genetic background	Major phenotype	Comments	Reference
Gpr54^{tm1PTL}	Gene targeting. Deletion of parts of exon 1 and 2	129S6/SvEv	Hypogonadotrophic hypogonadism	*Gpr54* expression tagged with *LacZ*, Vaginal opening not observed until greater than 100 days after birth	[26]
Gpr54^{tm1SPR}	Gene targeting. Deletion of part of exon 2	129P2/OlaHsd × C57Bl/6	Hypogonadotrophic hypogonadism	Similar phenotype on 10th generation backcross to C57Bl/6	[10]
Gpr54^{tm1HPC}	Gene targeting. Complete deletion of exon 2	129S1/SvImJ	Hypogonadotrophic hypogonadism	Most females show vaginal opening by 44 days after birth	[17]
Gpr54^{GtOmer}	Retroviral insertion intron 2. No loss of coding sequence	129S1/SvImJ	Hypogonadotrophic hypogonadism	No sexual dimorphism in AVPV region, females still show pre-ovulatory LH surge	[8,15]
Kiss1^{tm1PTL}	Gene targeting. Complete deletion of exon 1 and 2	129S6/SvEv	Hypogonadotrophic hypogonadism	*Kiss1* expression tagged with *LacZ*. Vaginal opening not observed until greater than 100 days after birth	[6]
Kiss1^{tm1HPC}	Gene targeting. Deletion of exon 1	129S1/SvImJ	Hypogonadotrophic hypogonadism	Most females show vaginal opening by 44 days after birth. Bimodal phenotype compared with other lines. Some females show larger gonadal weights, vaginal cornification and poor responses to kisspeptin injection. Some females have ovarian cysts	[17]

AVPV: anteroventral periventricular; ^{tm}: targetted mutation; ^{Gt}: gene trap mutation. ^{PTL}: Paradigm Therapeutics Ltd; ^{SPR}: Schering Plough Research; ^{HPC}: Harvard Partners Center; ^{Omer}: Omeros Corporation.