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# Animal models of pituitary neoplasia

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ABSTRACT

Pituitary neoplasias can occur as part of a complex inherited disorder, or more commonly as sporadic (non-familial) disease. Studies of the molecular and genetic mechanisms causing such pituitary tumours have identified dysregulation of >35 genes, with many revealed by studies in mice, rats and zebrafish. Strategies used to generate these animal models have included gene knockout, gene knockin and transgenic over-expression, as well as chemical mutagenesis and drug induction. These animal models provide an important resource for investigation of tissue-specific tumourigenic mechanisms, and evaluations of novel therapies, illustrated by studies into multiple endocrine neoplasia type 1 (MEN1), a hereditary syndrome in which ~30% of patients develop pituitary adenomas. This review describes animal models of pituitary neoplasia that have been generated, together with some recent advances in gene editing technologies, and an illustration of the use of the Men1 mouse as a pre clinical model for evaluating novel therapies.



## 1. Introduction

Pituitary tumours represent 10–15% of all intracranial tumours. The standardised incidence rate of pituitary tumours is ~4/1,00,000, with the higher incidence being in females (~5/1,00,000) (Tjornstrand et al., 2014; Raappana et al., 2010; Gittleman et al., 2014). Pituitary tumours may occur as part of hereditary syndromes (e.g. Multiple Endocrine Neoplasia Type 1 (MEN1) or Type 4 (MEN4)), or as an isolated (non-syndromic) disorder which may be inherited (e.g. Familial Isolated Pituitary Adenomas (FIPA)), or more commonly (>95%) as non-familial (i.e. sporadic) neoplasms (Yates et al., 2014). Pituitary tumours are also classified according to their hormonal production as: lactotrophinomas (secreting prolactin), that comprise ~50% of tumours; gonadotrophinomas (secreting follicle stimulating hormone (FSH) or lutenising hormone (LH), but predominantly non-functioning), comprising ~30% of tumours; somatotrophinomas (secreting growth hormone (GH)), comprising 15–20% of tumours; corticotrophinomas (secreting adrenocorticotropic hormone (ACTH)), comprising 5–10% of tumours; and thyrotrophinomas (secreting thyroid stimulating hormone (TSH)), comprising <1% of tumours. However, it is important to note that pituitary adenomas may secrete more than one hormone (Levy, 2008). Over 99% of pituitary tumours are benign adenomas with <0.2% being carcinomas that may metastasise (Scheithauer et al., 2006).

To date, over 30 animal models of pituitary tumourigenesis, usually mouse models, have been generated, using gene knockout and over-expression approaches. Evaluation of these models has increased our understanding of pituitary tumour biology and of the roles of oncogenic and tumour suppressor genes. This review will discuss these animal models of pituitary neoplasia, focussing on rodent models, together with the methods used in their generation. In addition, use of a *Men1* mouse model in evaluating approaches to targeted therapies will be reviewed.

## 2. Pituitary neoplasia models

Pituitary neoplasia may result from mutations involving either activation of a dominant gain-of-function oncogene, or inactivation of a recessive loss-of-function tumour suppressor gene. These mutations have been discovered by studies of pituitary tumours from patients, or from animal models generated for other disorders. To date, human studies of familial syndromes and sporadic disease have indicated the involvement of >35 genes in the development and progression of pituitary neoplasias (Table 1). Animal models harbouring mutations of ~35% of these genes have been generated, and animal models of mutations in genes not previously implicated in pituitary neoplasia have also been generated, such that over 40

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**Table 1**
Genetic abnormalities identified from human studies to be associated with pituitary neoplasias.

| Gene | Tumour type/Syndrome | Gene defect | Reference |
|---|---|---|---|
| *AIP* | FIPA<br>Young onset sporadic pituitary macroadenomas | Germline inactivating mutation | (Beckers et al., 2013) |
| *BMP-4* | Corticotrophinomas<br>Somatotrophinomas<br>Prolactinomas | Gene down-regulation<br>Gene over-expression<br>Gene over-expression | (Giacomini et al., 2006) |
| *CASP8* | Functioning and non-functioning adenomas | Methylation mediated gene silencing | (Bello et al., 2006) |
| *CCNA2* | MEN1 patients without *MEN1*, *CASR* or *HRPT2* mutations | Gene over-expression | (Agarwal et al., 2009) |
| *CCNB1* | MEN1 patients without *MEN1*, *CASR* or *HRPT2* mutations | Gene over-expression | (Agarwal et al., 2009) |
| *CCNB2* | MEN1 patients without *MEN1*, *CASR* or *HRPT2* mutations | Gene over-expression | (Agarwal et al., 2009) |
| *CCND1* | Non-functioning adenomas | Gene over-expression | (Jordan et al., 2000) |
| *CCNE1* | Cushing's syndrome<br>MEN1 patients without *MEN1*, *CASR* or *HRPT2* mutations | Gene over-expression | (Jordan et al., 2000; Jaffrain-Rea et al., 2013) |
| *CDH1* | Somatotrophinomas with prominent fibrous bodies | Methylation-mediated gene silencing | (Zhou et al., 2013) |
| *CDH13* | Functioning and non-functioning adenomas | Methylation-mediated gene silencing, correlating with tumour aggressiveness | (Qian et al., 2007) |
| *CDKN1A* | Functioning and non-functioning adenomas | Gene down-regulation | (Hiyama et al., 2002) |
| *CDKN1B* | MEN4 patients | Germline inactivating mutation | (Pellegata et al., 2006) |
| *CDKN2A* | Functioning and non-functioning adenomas | Methylation-mediated gene silencing | (Zhou et al., 2013) |
| *CDKN2B* | Functioning and non-functioning adenomas | Methylation-mediated gene silencing | (Zhou et al., 2013) |
| *CDKN2C* | Functioning and non-functioning adenomas | Methylation-mediated gene silencing | (Zhou et al., 2013) |
| *DAPK* family | Functioning and non-functioning adenomas | Loss of expression | (Simpson et al., 2002) |
| *FGFR2* | Functioning adenomas | Methylation-mediated gene silencing | (Zhu et al., 2007) |
| *FGFR4* | Functioning adenomas | Constitutively phosphorylated | (Ezzat et al., 2002) |
| *GADD45B* | Gonadotrophinoma | Gene silencing | (Michaelis et al., 2011) |
| *GADD45G* | Functioning, but more commonly in non-functioning adenomas | Gene silencing | (Zhou et al., 2013) |
| *GNAS* | Somatotrophinomas | Mutations detected | (Mantovani et al., 2010) |
| *HMGA-1* | Prolactinomas | Gene over-expression | (De Martino et al., 2009) |
| *HMGA-2* | Prolactinomas | Gene over-expression | (Fedele et al., 2006) |
| *LGALS3* | Lactotrophinomas<br>Corticotrophinomas | Gene over-expression | (Righi et al., 2010) |
| *MEG3* | Non-functioning adenomas | Methylation-mediated gene silencing | (Zhang et al., 2010) |
| *MEN1* | MEN1<br>Young onset sporadic pituitary adenomas | Inactivating mutations and gene deletions | (Thakker, 2010) |
| *MGMT* | Carcinomas | Methylation-mediated gene silencing | (Zhou et al., 2013) |
| *PLAGL1* | Non-functioning adenomas | Methylation-mediated gene silencing | (Pagotto et al., 2000) |
| *PRKAR1A* | Somatotrophinomas<br>Non-functioning adenomas | Gene down-regulation | (Kirschner, 2010) |
| *PTAG* | Adenomas (subtype not defined) | Methylation-mediated gene silencing | (Bahar et al., 2004) |
| *PTTG1* | Functioning and non-functioning adenomas | Gene over-expression | (Salehi et al., 2008) |
| *RAS* family | Functioning and non-functioning adenomas | Activating mutations | (Karga et al., 1992) |
| *RASSF1* | Functioning and non-functioning adenomas | Methylation-mediated gene silencing | (Qian et al., 2005) |
| *RASSF3* | Somatotrophinomas | Methylation-mediated gene silencing | (Peng et al., 2013) |
| *RB1* | Aggressive adenomas<br>Carcinoma | Rare inactivating mutations, methylation-mediated gene silencing | (Pei et al., 1995) |
| *SOCS1* | Somatotrophinomas<br>Corticotrophinomas<br>Non-functioning adenomas | Methylation-mediated gene silencing | (Buslei et al., 2006) |
| *SOX2* | Early onset pituitary adenomas | Rare gene deletion | (Alatzoglou et al., 2011) |
| *TP53* | Carcinoma | Rare inactivating mutations | (Tanizaki et al., 2007) |
| | Atypical corticotrophinoma | Rare inactivating mutation in one patient | (Kawashima et al., 2009) |
| *USP8* | Corticotroph adenomas | Dominant gain of function mutations | (Reincke et al., 2015; Jian et al., 2015) |

animal models of pituitary neoplasia have been generated, with the majority of these animal models being mutant mice (Table 2). Many of these models represent human syndromes e.g. MEN1 (Crabtree et al., 2001; Bertolino et al., 2003a; Biondi et al., 2002; Loffler et al., 2007a,b; Harding et al., 2009) and MEN4 (Kiyokawa et al., 1996; Nakayama et al., 1996; Fero et al., 1996), as well as representing a range of pituitary neoplasms that include hyperplasia, adenomas and carcinomas (Table 2). These pituitary tumours may secrete hormones such as prolactin, GH, ACTH, FSH, LH and TSH, or they may be non-secreting, which is also referred to as non-functioning adenomas (Table 2). These models have been generated using different methods, which will be briefly reviewed below.

## 2.1. Generation of animal models

Mutant animal models may be generated using: gene deletion (knockouts); over-expression by transgenic expression of wild type or mutant alleles; mutagenesis using chemicals e.g. *N*-ethyl-*N*-nitrosourea (ENU), or radiation; drugs e.g. long-term oestrogen treatment; and the breeding of animals with spontaneously arising abnormalities.

### 2.1.1. Gene deletion models

Gene deletion by homologous recombination (also referred to as knockout) is one of the most widely used methods to generate specific mouse models. Methods are based on modifying the gene of interest in embryonic stem (ES) cells (Fig. 1). In conventional knockout models, a vector construct comprising a plasmid or attenuated virus encoding a DNA sequence with homology to the target gene, but carrying a mutated base or bases resulting in loss of the protein, together with a positive selection marker (e.g. *Neo*), flanked by LoxP sites (allowing subsequent excision from selected

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