Abnormal responses of these receptors by their ligands have been reported in the pathogenesis of PHPT and SHPT. This review will focus on the animal models of parathyroid diseases and the associated genes have provided us with valuable information on the pathophysiology of parathyroid diseases. The application of these animal models is significant for the development of new therapies.

**KEYWORDS:** calcitriol, CaR, cinacalcet, CKD, FGF-23, hyperparathyroidism, Klotho, PTH

### INTRODUCTION

Hyperfunctioning parathyroid diseases such as primary hyperparathyroidism (PHPT) and secondary hyperparathyroidism of uremia (SHPT) are characterized by the abnormal metabolism of calcium (Ca) and phosphate (P). Parathyroid hormone (PTH), the active form of vitamin D (1,25-dihydroxyvitamin D, or 1,25(OH)\(_2\)D), and fibroblast growth factor (FGF)-23, are the principal physiological regulators of Ca and P homeostasis in humans (Imanishi et al., 2009a; Figure 1). There are feedback loops between ionized Ca (Ca\(^{2+}\)), P, 1,25(OH)\(_2\)D, FGF-23, and PTH.

Three receptors in parathyroid cells that are important in Ca and P homeostasis are the calcium-sensing receptor (CaR) and FGF receptor (FGFR-Klotho complex), which are located on the cell surface, and the vitamin D receptor (VDR) in the nucleus. Abnormal responses of these receptors by their ligands have been reported in the pathogenesis of PHPT and SHPT.

This review will focus on the animal models of parathyroid diseases, which exhibit abnormalities in Ca and P homeostasis (Table 1).

### RECEPTORS IN PARATHYROID CELLS

#### CALCIUM-SENSING RECEPTOR

Positional cloning approaches have clarified that loss-of-function mutations in the CaR gene cause familial hypocalciuric hypercalcemia (homozygous mutations) and neonatal severe hyperparathyroidism (homozygous mutations; Pollak et al., 1993). CaR has a crucial role in PTH secretion from parathyroid cells by sensing extracellular Ca\(^{2+}\) (Figure 2).

Homozygous knockout mice for CaR exhibited a similar phenotype of familial hypocalciuric hypercalcemia (Ho et al., 1995). Serum PTH levels were inappropriately elevated, but their parathyroid glands did not enlarge. Homozygous knockout mice had markedly elevated serum Ca, PTH, retarded growth, and premature death (Ho et al., 1995), symptoms that are concordant with human neonatal severe hyperparathyroidism. Double homozygous CaR- and PTH-deficient (CaR\(^{−/−}\)/PTH\(^{−/−}\)) mice were rescued from early lethality and skeletal abnormalities, and exhibited normocalcemia with undetectable serum PTH (Liu et al., 2011), indicating that normocalcemia in patients with neonatal severe hyperparathyroidism may lengthen their lifespan and normalize skeletal growth and development.

**VITAMIN D RECEPTOR**

1,25(OH)\(_2\)D\(_3\) is a steroid hormone that plays a crucial role in Ca and P homeostasis, which are mediated by the VDR. Hereditary hypocalcemic vitamin D-resistant rickets (HVDDR) is an autosomal recessive disorder, caused by inactivating mutations in the VDR gene, resulting in target tissue insensitivity to 1,25(OH)\(_2\)D\(_3\) (Haussler et al., 1998). VDR knockout mice exhibit hypocalcemia, hypophosphatemia, rickets, alopecia, and hyperparathyroidism with enlarged parathyroid glands, a phenotype that is similar to HVDDR (Yoshizawa et al., 1997). Tissue-specific ablation of VDR in parathyroid tissue exhibits decreased parathyroid CaR expression and a moderate increment in basal PTH levels. However, no significant abnormalities in PTH-Ca sigmoidal curves were observed (Meir et al., 2009), suggesting a limited role for VDR in parathyroid patho-physiology.

**FGF RECEPTOR-KLOTHO COMPLEX**

Klotho, which is expressed in the kidney, and in the pituitary and parathyroid glands, converts FGFR1, a canonical receptor for various FGFs, into a specific receptor for FGF-23 (Urakawa et al., 2006). FGF-23 null mice exhibit various senescence-like phenotypes such as a short lifespan, infertility, atrophy of
lymphopoietic and reproductive organs, decreased bone mineral density, and ectopic calcification, a phenotype that is similar to Klotho-deficient mice (Shimada et al., 2004), suggesting that FGF-23 signaling is Klotho dependent.

The parathyroid cells expressing Klotho and FGFR1 are responsive to FGF-23, both in vivo and in vitro (Ben-Dov et al., 2007). Reduced expressions of Klotho and FGFR1 in hyperplastic parathyroid glands from SHPT patients (Komaba et al., 2010), suggesting reduced signaling by FGF-23 to parathyroid cells, have a role in the development of SHPT. However, studies on Klotho expression in uremic animals show conflicting results (Canalejo et al., 2010; Hofman-Bang et al., 2010). Further studies are necessary to clarify the role of FGFR-Klotho signaling in uremic parathyroid glands.

**GENES IDENTIFIED IN PARATHYROID DISEASES**

**CYCLIN D1**

Cyclin D1 was identified from parathyroid adenomas which harbored a DNA rearrangement that separated the PTH gene’s 5’ flanking region from PTH coding exons, and the DNA recombined with cyclin D1 proto-oncogene (Arnold, 1993). The parathyroid tissue-specific enhancer in the PTH 5’ flanking region drives the cyclin D1 expression located downstream of the enhancer by the rearrangement (Mallya et al., 2010).

To define the role of cyclin D1 in parathyroid neoplasia, transgenic mice that overexpress the cyclin D1 oncogene in parathyroid glands were generated using a transgene that mimics the human PTH-cyclin D1 gene rearrangement (Imanishi et al., 2001). PTH-cyclin D1 transgenic mice not only developed abnormal parathyroid cell proliferation, but also developed biochemical hyperparathyroidism with characteristic abnormalities in bone. Specifically, the transgenic mice had an altered PTH-Ca relationship, which was shifted upward and to the right, and was steeper relative to that in the wild-type mice (Figure 2), due to reduced CaR expression in the parathyroid glands of transgenic animals.

Cinacalcet is an allosteric modulator that activates the CaR and inhibits PTH secretion from parathyroid cells. Administration of cinacalcet shifts the sigmoidal curve left in the PTH-Ca relationship (Figure 2), because cinacalcet increases the sensitivity of CaR to Ca in parathyroid cells. A single administration of cinacalcet significantly suppressed serum Ca levels in PTH-cyclin D1 transgenic mice with moderate biochemical hyperparathyroidism (Kawata et al., 2005). In older transgenic mice with advanced hyperparathyroidism caused by severe hypo-expression of CaR,
Transgenic mice expressing the RET proto-oncogene with an MEN2A mutation (cysteine 6343arginine) developed thyroid C-cell hyperplasia or medullary carcinoma (Kawai et al., 2000). Despite the widespread transgene expression, however, transgenic mice displayed a very peculiar tissue-restricted phenotype, such as mammary or parotid gland adenocarcinoma. The role of RET should be elucidated in the pathogenesis of parathyroid tumorigenesis.

**MEN1**

Multiple endocrine neoplasia type 1 (MEN1) is an autosomal dominant familial endocrine neoplasm syndrome characterized by tumors in parathyroid, enteropancreatic endocrine tissues, and the anterior pituitary. MEN1, encoding menin, is a tumor suppressor gene, contributing to a mutated cell’s selective advantage for growth through its bi-allelic inactivation. Menin interacts with Smad3 and enhance the TGF-β signaling pathway to inhibit cell proliferation (Kaji et al., 2001). Menin also interacts with histone modifying enzymes, transcription factors including nuclear receptors to suppress cell proliferation.

Mice with heterozygous deletion of the MEN1 gene exon 3–8 developed tumors involving pancreatic islets, and the parathyroid, thyroid, adrenal cortex, and pituitary, with loss of the wild-type MEN1 allele (Crabtree et al., 2001). Another mouse knockout model has been generated by deleting exon 3, and its heterozygous mice developed parathyroid adenomas and carcinomas, insulinomas, gastrinomas, glucagonomas, prolactinomas, or somatotrophinomas (Bertolino et al., 2003). All these features seem to be compatible with human MEN1 syndrome. Although mice with heterozygous MEN1 inactivation developed parathyroid neoplasia, hypercalcemia was not reported (Crabtree et al., 2001; Bertolino et al., 2003).

The mice with parathyroid-specific deletion of the MEN1 gene exhibited not only parathyroid neoplasia but also biochemical hyperparathyroidism such as hypercalcemia with elevated PTH concentration (Libutti et al., 2003). It is still unknown why only the mice with parathyroid-specific deletion of the MEN1 gene but not conventional MEN1 mice exhibited biochemical hyperparathyroidism.

**HRPT2**

Hyperparathyroidism-jaw tumor (HPT-JT) syndrome is a rare autosomal dominant disorder, characterized by cystic parathyroid tumors and fibro-osseous lesions of the mandible and maxilla. The gene responsible for HPT-JT encodes parafibromin, a ubiquitously expressed 531-amino acid protein (Carpten et al., 2002). The inactivated mutations were observed in the encoded parafibromin protein, suggesting the gene is a tumor suppressor.

To determine the role of parafibromin in parathyroid tumorigenesis, a transcription factor encoded by the Hrpt2 gene, conventional, and conditional knockout mice were generated (Wang et al., 2008). Homozygous knockout mice were embryonic lethal. Controlled deletion of the gene after embryonic day 8.5 resulted in apoptosis and growth retardation. Deletion of the gene in the adult led to severe cachexia and early death. These results revealed the important role of parafibromin in development and survival, but its role in parathyroid tumorigenesis is still unknown.
Recently, an MEN1-like recessive multiple endocrine neoplasia-like syndrome was identified (named MEN4) in rats and humans, which is due to mutations in the CDKN1B gene, encoding for p27kip1, a cyclin-dependent kinase (Cdk) inhibitor that regulates the transition of cells from G1 to S phase (Pellegrata et al., 2006). Mutated CDKN1B, encoding the p27kip1, was also identified in MENX rats with juvenile cataracts (Pellegrata et al., 2006). These rats exhibited neoplasia of multiple endocrine tissues such as pheochromocytoma, paraganglioma, thyroid medullary C-cell hyperplasia/neoplasia, adenoma of the anterior pituitary gland, and hyperplasia of the parathyroid gland (Fritz et al., 2002), which were compatible to human MEN4.

Interestingly, p27-null mice developed pituitary adenomas as the sole tumor phenotype, although the MENX rats developed a broader spectrum of neuroendocrine tumors (Pellegrata et al., 2006). The altered sensitivity to p27 loss in various tissues by species may lead to the altered tissue expression patterns and phenotypes.

CONCLUSION

In this review, the animal models exhibiting abnormal Ca and P homeostasis were discussed. Many kinds of animal models can be generated by manipulating genes relating to Ca and P homeostasis, and genes identified in parathyroid diseases. Uremic animals such as 5/6-nephrectomized rats are also good models for SHPT, which is not discussed in this review. These models are the best tool not only for understanding the pathogenesis of parathyroid diseases, but also for developing new therapies for these diseases.

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