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Review

The puzzles of the prokineticin 2 pathway in human reproduction

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ABSTRACT

Prokineticin, 1 (PROK1) and prokineticin 2 (PROK2), are two closely related proteins that were identified as the mammalian homologs of their two amphibian homologs, mamba intestinal toxin (MIT-1) and *Bv8*. MIT-1 was initially identified as a non-toxic constituent in the venom of the black mamba snake (*Dendroaspis polylepis*) (Joubert and Strydom, 1980) while *Bv8* was identified in the skin secretion of the toad, *Bombina variegate* (Mollay et al., 1999). All three homologs stimulate gastrointestinal motility thus accounting for their family name "prokineticins" (Schweitz et al., 1990, 1999). However, since its initial description, both PROK1 and PROK2 have been found to regulate a dazzling array of biological functions throughout the body. In particular, PROK1 acts as a potent angiogenic mitogen on endocrine vascular epithelium, thus earning its other name, Endocrine gland–vascular endothelial factor (EG–VEGF) (LeCouter et al., 2002). In contrast, the PROK2 signaling pathway is a critical regulator of olfactory bulb morphogenesis and sexual maturation in mammals and this function is the focus of this review.

Contents

1. The prokineticin signaling pathway in humans

The mature human PROK1 peptide consists of 86 amino acids and is encoded by a three-exon gene on chromosome 1 (NCBI gene ID: 84432) while the most active PROK2 peptide consists of 81 amino acids and is encoded by a four-exon gene on chromosome 3 (NCBI gene ID: 60675). The additional exon of the PROK2 gene can be

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alternatively spliced, resulting in a longer isoform, PROK2L (102 amino acids) whose function is not well-understood (Wechselberger et al., 1999; Chen et al., 2005). The ligands, PROK1 and PROK2, show only 45% sequence homology but do share two conserved features essential for their bioactivity: a highly conserved hexapeptide "AVITGA" sequence at their N-terminal and a distinctive structural motif consisting of 10 cysteine residues with disulfide cross-linking. Although both PROK1 and PROK2 are co-expressed in various tissues including brain, ovary, testis, placenta, adrenal cortex, peripheral blood cells, intestinal tract, heart, and bone marrow (Ngan and Tam, 2008; Negri et al., 2009), there are some striking differential tissue expression patterns. For example, PROK1 is predominantly expressed in steroidogenic endocrine organs (LeCouter et al., 2001), whereas PROK2 is mainly expressed in the non-steroidogenic cells of the testes and the central nervous system. Also, PROK2 has a distinct rhythmic circardian expression in the suprachiasmatic nucleus and throughout the hypothalamus, key for its reproductive status. (Ferrara et al., 2004; Cheng et al., 2005).

Both prokineticins act as effective ligands for a pair of G-protein coupled receptors, prokineticin receptor 1 (PROKR1) and 2 (PROKR2). Although the human genes encoding these receptors are on two different chromosomes (*PROKR1* gene: 2p13.3; *PROKR2* gene: 20p13), the sequences of both receptors are remarkably conserved, displaying nearly 85% identity (Li et al., 2001; Masuda et al., 2002; Soga et al., 2002). Both ligands, PROK1 and the 2 PROK2 isoforms (PROK2 and PROK2L), bind and activate both PROK receptors in nanomolar range although PROK2 has a slightly higher affinity for both receptors (Lin et al., 2002; Masuda et al., 2002; Soga et al., 2002). However, the striking differential anatomical expression patterns of these receptors relates to their diverse biological actions. PROKR2 is abundantly expressed in the brain (olfactory bulb, subventricular zone, preoptic area, the paraventricular nucleus, the arcuate nucleus, and the median eminence) and testes, while PROKR1 is mainly expressed in peripheral tissues such as spleen, prostate, pancreas, heart and blood cells.

2. Prok2 pathway and its link to reproduction

2.1. Prok2/Prokr2 knockout mice: the first murine model of Kallmann Syndrome (KS)

Kallmann Syndrome (KS) is a severe neurodevelopmental phenotype resulting from the combined failure of neuronal migration of the olfactory and GnRH neuronal precursors (Seminara et al., 1998). Although the *KAL1* gene was the first human gene linked to the neurodevelopmental phenotype of KS (see Chapter 2), the mouse homolog of *kal1* is yet to be identified and hence a murine model for KS has remained elusive. A prime role for the PROK2/PROKR2 signaling pathway in the neuroendocrine control of mammalian reproduction was thus incidentally discovered when complete murine knockouts of *prok2* and *prokr2* mirrored the human phenotype of Kallmann Syndrome (KS) (Ng et al., 2005; Matsumoto et al., 2006). Given the initial focus on the primary gastrointestinal role assumed for the prokineticins, this finding was completely unanticipated. Both *prok2* and *prokr2* knockout mice showed disruption of the neurogenesis of their olfactory bulbs (OB) (Ng et al., 2005; Matsumoto et al., 2006) accompanied by a dramatic reduction of the GnRH-expressing cells in the median preoptic area as well as absence of GnRH neural projections in the medium eminence (Pitteloud et al., 2007). These findings were a phenocopy of the anatomical observation of KS in humans, specifically the arrest of their GnRH neuronal migration. These arrested GnRH neurons in the mouse knock outs formed a 'fibrocellular mass' just beyond the cribiform plate immediately prior to their entry into the forebrain (Matsumoto et al., 2006; Pitteloud et al., 2007).

This reduction of hypothalamic GnRH neurons in *prok2* and *prokr2* knockout mice results in failure of GnRH secretion and as a consequence, both male and female knockout mice display hypogonadotropism and impairment of sexual development. Male *prok2* and *prokr2* knockout mice show small seminiferous tubules that lack lumens, absent haploid spermatocytes and spermatids (Pitteloud et al., 2007). Similarly, female *prok2* and *prokr2* knockout mice show incomplete follicular development characterized by absence of mature follicles and corpora lutea (Matsumoto et al., 2006; Pitteloud et al., 2007). Although the reproductive phenotype of the *prok2* and *prokr2* knockout mice are remarkably similar, a major difference between the *prok2* and *prokr2* knockout mice is seen in the olfactory system development. While almost all *prokr2* knockout mice show hypoplasia/agenesis of both olfactory bulbs, (Matsumoto et al., 2006), approximately half of the *prok2* knockout mice exhibit asymmetric olfactory bulb development (Pitteloud et al., 2007), suggesting potential redundancy between the two ligands, PROK1 and PROK2, in the neurogenesis of the OB.

2.2. PROK2 and PROKR2 mutations in isolated GnRH deficiency in humans

Following these findings in murine knockouts of *prok2* and *prokr2,* the prokineticin 2 pathway became an obvious candidate gene to test for the etiology of human GnRH deficiency. In 2006 Dode et al. (2006), screened 192 unrelated KS patients and reported several DNA sequence changes in both *PROK2* and *PROKR2* without any functional studies in the missense cases. However, in contrast to the murine knock outs, the majority of these rare sequence variants associated with the clinical phenotype were discovered to exist only in the heterozygous state (four patients with heterozygous mutations in *PROK2* and 10 patients with heterozygous *PROKR2* variants). Homozygous or compound heterozygous changes were seen in only four subjects. Subsequently, Pitteloud et al., 2007) reported three affected siblings with GnRH deficiency (two brothers and one sister of Portuguese ethnicity) all of whom harbored a loss-of-function homozygous deletion in the ligand, *PROK2,* that resulted in a biologically inactive 27 amino acid truncated protein. Following these initial reports, a large number of predominantly heterozygous loss-of-function mutations in both *PROK2* (Fig. 1) and *PROKR2* (Fig. 2) have now been reported in patients with both KS and nIHH by several independent groups (Sarfati et al., 2010; Cole et al., 2008; Leroy et al., 2008; Sinisi et al., 2008; Canto et al., 2009; Monnier et al., 2009). Thus, although both the human and mouse studies have confirmed and firmly established a key role of the PROK2 pathway in mammalian reproduction, several features of this biology remain puzzling, suggesting a more complex systems biology of this pathway in humans. These puzzles are detailed below and the underlying basis for these intriguing observations is yet to be fully ascertained.

3. The puzzles of the prokineticin pathways

The study of humans with mutations PROK2 pathway have greatly helped expand the initial murine observations. However, strikingly, the combined analysis of the murine and human phenotypes have raised several puzzling observations. First, despite the key neurodevelopmental role of the PROK2 pathway, the PROKR2 receptor is conspicuously absent on both developmental and mature adult GnRH neurons. Second, in contrast to a pure 'neurodevelopmental' phenotype in mice, i.e. a combination of olfactory and reproductive phenotypes, humans with *PROK2/PROKR2* mutations present with both KS as well as normosmic idiopathic hypogonadotropic hypogonadism (nIHH). This observation indicates that the PROK2 pathway plays a key role in both the neurodevelopmental