Title

The Prokineticins: Neuromodulators and Mediators of Inflammation and Myeloid Cell-Dependent Angiogenesis

Journal

Physiological Reviews, 98(2)

ISSN

0031-9333

Authors

Negri, Lucia
Ferrara, Napoleone

Publication Date

2018-04-01

DOI

10.1152/physrev.00012.2017

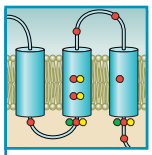
Peer reviewed

Physiol Rev 98: 1055–1082, 2018
Published March 14, 2018; doi:10.1152/physrev.00012.2017

THE PROKINETICINS: NEUROMODULATORS AND MEDIATORS OF INFLAMMATION AND MYELOID CELL-DEPENDENT ANGIOGENESIS

Lucia Negri and Napoleone Ferrara

Sapienza University of Rome, Rome, Italy; and University of California, San Diego, La Jolla, California



Negri L, Ferrara N. The Prokineticins: Neuromodulators and Mediators of Inflammation and Myeloid Cell-Dependent Angiogenesis. *Physiol Rev* 98: 1055–1082, 2018. Published March 14, 2018; doi:10.1152/physrev.00012.2017.—The mammalian prokineticins family comprises two conserved proteins, EG-VEGF/PROK1 and Bv8/PROK2, and their two highly related G protein-coupled receptors, PKR1 and PKR2. This signaling system has been linked to several important biological functions, including gastrointestinal tract motility, regulation of circadian rhythms, neurogenesis, angiogenesis and cancer progression, hematopoiesis, and nociception. Mutations in *PKR2* or *Bv8/PROK2* have been associated with Kallmann syndrome, a developmental disorder characterized by defective olfactory bulb neurogenesis, impaired development of gonadotropin-releasing hormone neurons, and infertility. Also, Bv8/PROK2 is strongly upregulated in neutrophils and other inflammatory cells in response to granulocyte-colony stimulating factor or other myeloid growth factors and functions as a pronociceptive mediator in inflamed tissues as well as a regulator of myeloid cell-dependent tumor angiogenesis. Bv8/PROK2 has been also implicated in neuropathic pain. Anti-Bv8/PROK2 antibodies or small molecule PKR inhibitors ameliorate pain arising from tissue injury and inhibit angiogenesis and inflammation associated with tumors or some autoimmune disorders.

I. HISTORICAL NOTE ON THE DISCOVERY OF PROKINETICINS

The complex history of this family of small proteins began about 40 years ago, when Strydom (218) isolated multiple proteins from black mamba (*Dendroaspis polylepis polylepis*) venom. Among these, "venom protein A" or "VPRA" seemed devoid of toxic effects in standard tests (218). A few years later, the same group determined the amino acid sequence of VPRA and noted the lack of homology with any known black mamba protein (109). In 1999, Schweitz et al. (198) purified a protein from black mamba venom that stimulated contraction of the guinea pig ileum, hence the name "mamba intestinal toxin 1" (MIT-1). Sequence analysis revealed that MIT-1 was the same protein as VPRA (198). The same group also described a key structural feature of this protein, the existence of a "colipase fold" (17).

In 1999 Mollay et al. (158) isolated from the skin secretion of the frog *Bombina variegata* a small protein, which was closely related to VPRA/MIT-1 (58% sequence identity), and named it "Bv8" to indicate its origin (*B. variegata*) and molecular weight (8 kDa). Similar to MIT-1, Bv8 contracted guinea pig, rat, and mouse ileum and relaxed rat colon. Additionally, they reported that Bv8 induced hyperalgesia in rats (158).

In 2001, Li et al. (136), searching EST databases for homologies to the predicted coding region of Bv8, identified two human sequences, one encoding a protein of 86 amino acids which showed a high degree of homology with VPRA/MIT1 and the other encoding an 81-amino acid protein highly related to Bv8. Similar to VPRA and Bv8, these proteins potently stimulated the contraction of gastrointestinal (GI) smooth muscle. To reflect this motility-enhancing activity, the two human proteins were named respectively prokineticin 1 (PROK1) and prokineticin 2 (PROK2) (136).

At about the same time, LeCouter et al. (127) reported the identification of a molecule capable of stimulating proliferation of adrenal cortex-derived endothelial cells from screening a library of human secreted proteins (42). They expected this molecule to be a member of a known family of endothelial cell mitogens, but surprisingly, it displayed a remarkably high degree of homology (80%) with VPRA/MIT1 (126, 127). Based on its selective expression in human steroidogenic organs, this protein was named endocrine gland-derived vascular endothelial growth factor (EG-VEGF) (127). These early studies showed that EG-VEGF

induced proliferation, migration, and fenestration in endothelial cells in vitro and a strong angiogenic response when delivered in the rat ovary. Similar to VEGF-A (70), the expression of EG-VEGF was induced by hypoxia, through a hypoxia-inducible factor-dependent mechanism (127). The mature human EG-VEGF protein proved identical to PROK1 (126, 127, 136).

While the nomenclature can be quite confusing given the multiple names of the mammalian and nonmammalian proteins, it is now well established that this family comprises only two ligand members, which are encoded by two distinct genes. EG-VEGF/PROK1 is the ortholog of VRPA/MIT1, while Bv8/PROK2 is the ortholog of Bv8.

II. STRUCTURE, PHYLOGENESIS, AND HOMOLOGIES OF PROKINETICINS

Mammalian and nonmammalian prokineticins exhibit an identical amino terminal sequence, AVITGA, which is critical for their biological activity. On this basis, Kaser et al. (110) proposed the general term *AVIT proteins* to designate this family. Substitutions, deletions, or insertions to this hexapeptide result in the loss of agonist activity on prokineticin receptors (23, 166).

The presence of 10 cysteines with identical spacing defines a 5-disulfide bridge that confers to the molecule a compact structure with NH_2 and COOH ends present at the surface. Several charged residues are buried inside the molecule, whereas some hydrophobic residues, such as Trp24, are exposed on the surface. Substituting Trp in position 24 with Ala changes the relative affinity and efficacy of Bv8 for the receptors (124). One side of the roughly ellipsoid protein has a positive net charge, whereas the opposite side is hydrophobic (110). A similar Cys motif is also present in mammalian colipase [called a colipase fold, as already noted (17)] and in the COOH-terminal region of members of the Dickkopf family of extracellular signaling proteins that organize head development in embryos (85). Prokineticins, however, do not stimulate the activity of pancreatic lipase and are also inactive in an assay measuring Dickkopf functions (17, 110, 223). Homologs of Bv8 have been identified in skin secretions of various amphibians, *Bombina bombina*, *Bombina orientalis* (Bo8), and *Bombina maxima* (Bm8a) (33), and also in lizards and in Takifugu fish species (110) **(FIGURE 1)**. Also, groups of proteins that share the colipase fold and some sequence identity and homology with MIT-Bv8-prokineticins have been identified in the venom of Australian funnel-web spiders *Hadronyche sp*. (223, 239), in the cnidarian allomonal system (202), and in hemocytes of crayfish. Interestingly, these crustacean proteins, astakines, are produced by hemocytes and are released into the plasma. Astakines are among the first invertebrate cytokines shown to be involved in hematopoiesis, and thus they may provide new evolutionary perspectives about this process (210). However, it has been reported that these invertebrate homologs do not activate the mammalian prokineticin receptors due to the lack of the six conserved NH_2-terminal amino acids, making their inclusion in the prokineticin family questionable. Subsequent studies reported that astakine binds plasma membrane ATP synthase (141), although the signaling receptor for astakine is presently unknown.

In murine and human testis, two forms of Bv8-like proteins are present, containing 81 and 102 amino acids, respectively **(FIGURE 2)**. They differ in the sequences encoded by an exon, 21 residues of which 19 are basic (lysines or arginines) occurring in two clusters, raising the possibility that some proteolytic cleavage could take place as these molecules cross the secretory pathway, possibly generating a short molecule (PK2β) that retains the receptor binding and activation domain (32). However, it remains to be determined whether prokineticins can undergo proteolytic cleavage by extracellular proteases in vivo. The long form is identified as mBv8b or $Bv8_{102}$ or PROK2L (130, 238). The transcript lacking the insert, indicated as Bv8a or $Bv8_{81}$ or PROK2, similar to Bv8 from frog, is present in many organs. The longer form, however, only could be detected unambiguously in murine and human testis, in primary spermatocytes, and in rat and mouse macrophages (84, 150). A third transcript with a portion of intron 2, that would yield a truncated form of Bv8 lacking both the basic stretch and the COOH-terminal part, has been found in mouse brain (108). The recently described amino acid sequence of simian (*Macaca mulatta*) PROK2 (24) displays only two conservative substitutions compared with human PROK2 sequences (V51F, R80Q) and three conservative substitutions compared with mouse PROK2 sequences (K36Q, L37V, W51V).

The genomic structure of murine and human Bv8/PROK2 genes has been determined, and the chromosomal localization was identified near a synteny breakpoint of mouse chromosome 6 and human 3p21, located within an unstable chromosomal region. Exon 1 encodes the signal peptide and the first five amino acids of the mature protein. Exon 2 encodes 42 amino acids, including 6 of the 10 cysteines of the mature protein. Exon 3 encodes the 21-amino acid insert that is present in an alternative mRNA. The remaining 34 amino acids are encoded by exon 4, including 4 of the 10 cysteines of the secreted protein. The Bv8 human and mouse promoter sequences are highly conserved (108, 130). **FIGURE 3** illustrates the genomic structure and schematic protein sequences of Bv8/PROK2 and EG-VEGF/PROK1.

As noted, EG-VEGF/PROK1 is most closely related to VPRA/MIT-1 (83% homology) and shows only 45% sequence homology with PROK2/Bv8. Mouse EG-VEGF maps to a region of chromosome 3 synthenic with human chromosome 1p13.1, the locus for human EG-VEGF/PROK1. The gene organization is highly conserved, com-

Bv8-related peptides in frogs, fishes, lizards and snakes

Peptide	Primary structure	Species
		Amphibians
	1----------38	
Bv8	AVITGA**C**DKDVQ**C**GSGT**CC**AASAWSRNIRF**C**IPLGNSG	Bombina variegata
Bm8a	AVITGV**C**DRDAQ**C**GSGT**CC**AASAFSRNIRF**C**VPLGNNG	Bombina maxima
Bm8b	AVITGVRDRDAQ**C**GSGT**CC**AASAFSRNIRF**C**VPLGNNG	Bombina maxima
Bm8c	AVITGV**C**DRDAQ**C**GSGT**CC**AASAFSRNVRF**C**VPLGNNG	Bombina maxima
Bm8d	AVITGV**C**DRDAQ**C**GSGT**CC**AASAFSRNIRF**C**VPLGNNG	Bombina maxima
Bm8e	AVITGV**C**DRDAQ**C**GSGT**CC**AASAFSRNIRF**C**VPLGNNG	Bombina maxima
Bm8f	AVITGV**C**DRDAQ**C**GSGT**CC**AASAFSRNIRF**C**VPLGNNG	Bombina maxima
Bo8	AVITGA**C**DRDVQ**C**GSGT**CC**AASAWSRNIRF**C**VPLGNSG	Bombina orientalis
	39----------77	
Bv8	ED**C**HPASHKVPYDGKRLSSL**C**P**C**KSGLT**C**SKSGEKFK**C**S	Bmbina variegata
Bm8a	EE**C**HPASHKVPYNGKRLSSL**C**P**C**NTGLT**C**SKSGEKFQ**C**S	Bombina maxima
Bm8b	EE**C**HPASHKVPYNGKRLSSL**C**P**C**NTGLT**C**SKSGEKYQ**C**S	Bombina maxima
Bm8c	EE**C**HPASHKVPYNGKRLSSL**C**P**C**NTGLT**C**SKSGEKFQ**C**S	Bombina maxima
Bm8d	EE**C**HPASHKVPYNGKRLSSL**C**P**C**NTGLT**C**SKSGEKSQ**C**S	Bombina maxima
Bm8e	EE**C**HPASHKVPYNGKRLSSL**C**P**C**NTGLT**C**PKSGEKFQ**C**S	Bombina maxima
Bm8f	EE**C**HPASHKVPSDGKRLSSL**C**P**C**NTGLT**C**SKSGEKYQ**C**S	Bombina maxima
Bo8	EE**C**HPASHKVPYDGKRLSSL**C**P**C**KSGLT**C**SKSGAKFK**C**S	Bombina orientalis
		Fishes
	1----------38	
Fugu-1	AVITGA**C**ERDVQ**C**GLGL**CC**AVSLWLRGLRM**C**APRGLEG	Takifugu bimaculatus
Fugu-2	AVITGA**C**EKDSQ**C**GGGM**CC**AVSLWIRSLRM**C**TPMGREG	Takifugu chinensis
	39----------77	
Fugu-1	DE**C**HPFSHKVPYPGKRQHHT**C**P**C**LPHLV**C**TRDRDSKYR**C**	Takifugu bimaculatus
Fugu-2	DD**C**HPMSHTVPFFGKRLHHT**C**P**C**LPNLS**C**IPMDEGRAK**C**	Takifugu chinensis
	78----------94	
Fugu-1	TDDFKNVDLYEVGQTLR	Takifugu bimaculatus
Fugu-2	LSTYKYPDYYL	Takifugu chinensis
		Snakes
	1----------38	
MIT-1	AVITGA**C**ERDLQ**C**GKGT**CC**AVSLWIKSVRV**C**TPVGTSGE	Dendroaspis polylepsis
	39----------77	
MIT-1	D**C**HPASHKIPFSGQRKMHHT**C**P**C**APNLA**C**VQTSPKKFK**C**	Dendroaspis polylepsis
	-80	
MIT-1	LSK	Dendroaspis polylepsis
		Lizards
	1----------38	
VAR-1	AVITGA**C**DKDLQ**C**GEGM**CC**AVSLWIRSIRI**C**TPLGSSGE	Varanus varius
VAR-2	AVITGA**C**DKDLQ**C**GEGM**CC**AVSLWIRSIRI**C**TPLGSSGE	Varanus varius
	39----------77	
VAR-1	D**C**HPLSHKVPFDGQRKHHT**C**P**C**LPNLV**C**GQTSPGKYK**C**L	Varanus varius
VAR-2	D**C**HPLSHKVPFDGQRKHHT**C**P**C**LPNLV**C**GQTSPGKHK**C**L	Varanus varius
	78---84	
VAR-1	PEFKNVF	Varanus varius
VAR-2	PEFKNVF	Varanus varius

FIGURE 1. Amino acid sequences of non-mammalian prokineticins. The AVITG motif in the NH_2-terminal region of the mature protein, required for biological activity, and the cysteine residues, required for the "colipase fold," are highlighted.

Bv8-related prokineticins in mammals

Peptides	Primary structure	Species
	1----------45	
mPK1	AVITGA**C**ERDIQ**C**GAGT**CC**AISLWLRGLRL**C**TPLGREGEE**C**HPGS	mouse
mPK2	AVITGA**C**DKDSQ**C**GGGM**CC**AVSIWVKSIRI**C**TPMGQVGDS**C**HPLT	mouse
mPK2b	AVITGA**C**DKDSQ**C**GGGM**CC**AVSIWVKSIRI**C**TPMGQVGDS**C**HPLT	mouse
rPK1	AVITGA**C**ERDVQ**C**GAGT**CC**AISLWLRGLRL**C**TPLGREGEE**C**HPGS	rat
rPK2	AVITGA**C**DKDSQ**C**GGGM**CC**AVSIWVKSIRI**C**TPMGQVGDS**C**HPLT	rat
rPK2b	AVITGA**C**DKDSQ**C**GGGM**CC**AVSIWVKSIRI**C**TPMGQVGDS**C**HPLT	
bPK1	AVITGA**C**ERDVQ**C**RAGT**CC**AVSLWLRGLRV**C**TPLGRAGEE**C**HPGS	cattle
bPK2	AVITGA**C**DRDPQ**C**GGGM**CC**AVSLWVKSIRI**C**TPMGKVGDS**C**HPMT	cattle
bPK2b	AVITGA**C**DRDPQ**C**GGGM**CC**AVSLWVKSIRI**C**TPMGKVGDS**C**HPMT	cattle
mkPK2	AVITGA**C**DKDSQ**C**GGGM**CC**AVSIWVKSIRI**C**TPMGKLGDS**C**HPLT	monkey
mkPK2b	AVITGA**C**DKDSQ**C**GGGM**CC**AVSIWVKSIRI**C**TPMGKLGDS**C**HPLT	monkey
hPK1	AVITGA**C**ERDVQ**C**GAGT**CC**AISLWLRGLRM**C**TPLGREGEE**C**HPGS	man
hPK2	AVITGA**C**DKDSQ**C**GGGM**CC**AVSIWVKSIRI**C**TPMGKLGDS**C**HPLT	man
hPK2b	AVITGA**C**DKDSQ**C**GGGM**CC**AVSIWVKSIRI**C**TPMGKLGDS HPLT	man
	46----------86	
mPK1	HKIPFLRKRQHHT**C**P**C**SPSLL**C**SRFPDGRYR**C**FRDLKNANF	mouse
mPK2	RKVPFWGRRMHHT**C**P**C**LPGLA**C**LRTSFNRFI**C**LARK	mouse
mPK2b	RKSHVANGRQERRRAKRRKRKKEVPFWGRRMHHT**C**P**C**LPGLA**C**LRTSFNRFI**C**LARK	mouse
rPK1	HKIPFFRKRQHHT**C**P**C**SPSLL**C**SRFPDGRYR**C**SQDLKNVNF	rat
rPK2	RKVPFWGRRMHHT**C**P**C**LPGLA**C**LRTSFNRFI**C**LARK	rat
rPK2b	RKSHVANGRQERRRAKRRKRKKEVPFWGRRMHHT**C**P**C**LPGLA**C**LRTSFNRFI**C**LARK	rat
bPK1	HKVPFFRKRQHHA**C**P**C**LPNLL**C**SRGLDGRYR**C**STNLKNINF	cattle
bPK2	RKVPFLGRRMHHT**C**P**C**LPGLA**C**SRTSFNRYT**C**LAQK	cattle
bPK2b	RKNHFGNGRQERRKRKRRRKKKVPFLGRRMHHT**C**P**C**LPGLA**C**SRTSFNRYT**C**LAQK	cattle
mkPK2	RKVPFLGRRMHHT**C**P**C**LPGLA**C**LRTSFNRFI**C**LARK	monkey
mkPK2b	RKNNFGNGRQERRKRKRRKRKKEVPFGRRMHHT**C**P**C**LPGLA**C**LRTSFNRFI**C**LARK	monkey
hPK1	HKIPFFRKRKHHT**C**P**C**LPNLL**C**SRFPDGRYR**C**SMDLKNINF	man
hPK2	RKVPFFGRRMHHT**C**P**C**LPGLA**C**LRTSFNRFI**C**LAQK	man
hPK2b	RKNNFGNGRQERRKRKRSKRKKEVPFFGRRMHHT**C**P**C**LPGLA**C**LRTSFNRFI**C**LAQK	man

FIGURE 2. Amino acid sequences of mammalian prokineticins. PK1, EG-VEGF/PROK1; PK2, Bv8/PROK2. Highlighted are the AVITG motif, the cysteine residues, and the highly basic, alternative spliced, sequence encoded by exon 3 in PK2b.

posed of three exons, with no known alternative splicing product. But the promoter sequences have diverged; for example, the human *EG-VEGF/PROK1* promoter has a potential binding site for an orphan nuclear receptor essential for adrenal development [steroidogenic-factor 1 (SF-1), or NR5A1] (177). Indeed, as already pointed out, in human and non-human primates, EG-VEGF/PROK1 expression is restricted to steroidogenic cells, with ovary and testis expressing the highest level of transcript, whereas mouse EG-VEGF transcript is restricted predominantly to the liver and kidney (129). Surprisingly, in the bovine tissues, the pattern of EG-VEGF/PROK1 and Bv8/PROK2 expression appears closer to the distribution in the mouse than in the human tissues: EG-VEGF/PROK1 is more abundantly expressed in liver than in endocrine tissues (112).

Regulators for the prokineticins have been described in reproductive tract, neurons, and macrophages. In the reproductive tract, the expression of EG-VEGF/PROK1 may be upregulated by estrogen, progesterone, and human chorionic gonadotropin, as well as hypoxia-inducible factor-1a (127, 172, 173, 226). In olfactory bulbs, the expression of Bv8/PROK2 is elevated by two proneural basic helix-loop-helix factors (neurogenin1 and Mash1) and repressed by homeobox transcrip-

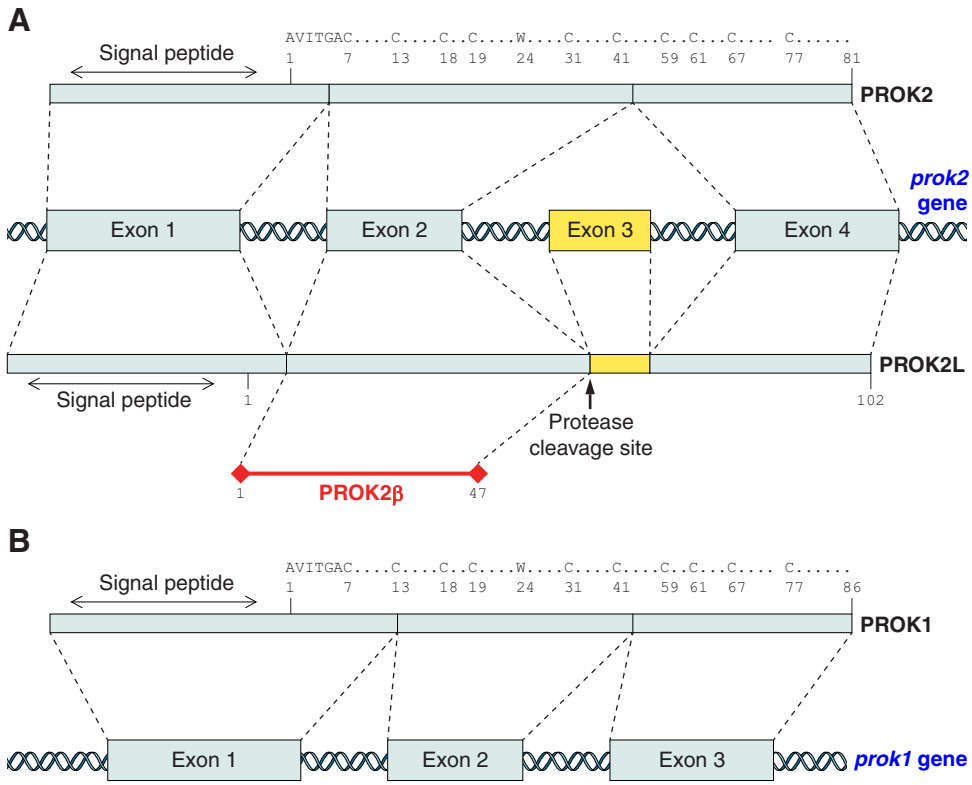


FIGURE 3. Genomic structure and schematic protein sequences of Bv8/PROK2 (*A*) and EG-VEGF/PROK1 (*B*). *Bv8/PROK2* gene has been localized near a synteny breakpoint of mouse chromosome 6 and human 3p21, located within an instable chromosomal region. As indicated in the text, exon 1 encodes the signal peptide and the first 5 amino acids of the mature protein; exon 2 encodes 42 amino acids, including 6 of the 10 cysteines of the mature protein, and exon 3 encodes the 21-amino acid insert that is present in an alternative mRNA. The remaining 34 amino acids are encoded by exon 4, including 4 of the 10 cysteines of the mature protein. The *Bv8/PROK2* human and mouse promoter sequences are highly conserved, indicating a related transcriptional regulation in these species (108, 130). Mouse *eg-vegf/prok1* maps to a region of chromosome 3 synthenic with human chromosome 1p13.1, the locus for human *EG-VEGF/PROK1*. The gene organization is highly conserved, composed of three exons, with no known alternative splicing product. However, the promoter sequences have diverged among species (see text) leading to different localization: in steroidogenic glands in human and non-human primates (177), in liver and kidney in mouse (129), and in liver in bovine (112).

tional factors (distal-less homeobox 1 and 2) (247). Bv8/PROK2 is positively regulated in neutrophils and bone marrow, specifically by granulocyte colony stimulating factor (207) but by interleukin (IL)-10 in human monocytes and lymphocytes. Granulocyte-colony stimulating factor (G-CSF) may be also responsible for Bv8/PROK2 upregulation in sensitive neurons and in central nervous system (CNS) after nerve damage or neuroinflammation (1, 143).

Prokineticin variants have been found in humans. EG-VEGF/PROK1-V67I occurs in nearly 50% of the general population and is usually considered as a neutral variant, not directly implicated in recurrent miscarriage. However, it has been reported to confer a modifier effect on early pregnancy through interaction with PKR1 and PKR2 variants (219). So far, 10 *Bv8/PROK2* mutations have been found in a minority of Kallmann syndrome (KS) patients, most heterozygotes. Most of these mutations may impair correct synthesis and/or activity of the ligand (55).

It has been pointed out that the prokineticin family shares several common features with the chemokine superfamily, such as their small size (8 kDa), receptors (G protein-coupled receptors, GPCR), signaling mechanisms, as well as chemotactic and immune-modulatory activities (161). However, a key structural difference is the presence of 10 cysteine residues in prokineticins, whereas chemokines contain 4–6 cysteine residues. A phylogenetic study to compare the degree of similarity among prokineticins, chemokines, and defensins, a subclass of cationic antimicrobial peptides involved in innate immunity, revealed a higher similarity of amino acid sequence between defensins and prokineticins than with chemokines (161).

III. DIFFERENTIAL EXPRESSION OF PROKINETICINS

Although both EG-VEGF/PROK1 and Bv8/PROK2 are co-expressed in various tissues, there are some striking differ-

ences in tissue expression patterns (see **TABLE 1**). A major difference is that, in humans, *EG-VEGF/PROK1* is predominantly expressed in steroidogenic endocrine glands (127), whereas *Bv8/PROK2* is mainly expressed in the non-steroidogenic cells.

Mammalian testis expresses the highest level of prokineticins. *EG-VEGF/PROK1* is restricted to the testosterone-producing Leydig cells, whereas *Bv8/PROK2* is expressed in the seminiferous tubules and restricted to the primary spermatocytes (130, 194, 238).

In the rat gut, *Eg-vegf/prok1* mRNA expression is significantly higher in the stomach than in the small intestine, especially in fundus where it is 70-fold higher than *Bv8/prok2* mRNA (234). *Eg-vegf/prok1* (but not *bv8/prok2*) is

Table 1. *Expression pattern of PROK1, PROK2, PROKR1, and PROKR2 in mouse and human tissues*

TISSUES	PROK1	PROK2	PKR1	PKR2	Reference Nos.
Brain	Tractus solitarium, cerebellum	Olfactory bulb, SCN, medial preoptic area, nucleus arcuatus, nucleus accumbens, Calleja islands, medial amygdala, mesencephalon, cerebral cortex, cerebellum	Embryo ventricles olfactory bodies, nucleus arcuatus, mammillary bodies, hypothalamus, mesencephalon, Gasser ganglion	Embryo ventricles, olfactory bodies, piriform and entorhinal cortex, lateral preoptic area, hippocampus, globus pallidus, amygdala, SCN, paraventricular nucleus, nucleus arcuatus, median eminence, mammillary nucleus, subfornical organ, Gasser ganglion	35, 155, 168
Spinal cord		Astrocytes, central endings of nociceptors	Astrocytes, central endings of nociceptors	Projection neurons and motor neurons	143
Peripheral nervous system		DRG: some TRPV1+ neurons, Schwann cells	DRG: mainly inTRPV1+ neurons	DRG: mainly TRPA1+ neurons	165
Pituitary				Neuropituitary	138, 151
Ovary	Granulosa, theca cells		Capillary endothelial cells	Capillary endothelial cells	69, 73, 127, 138
Uterus	Glandular epithelium, stromal and smooth muscle cells	Glandular epithelium, stromal and smooth muscle cells	Glandular epithelium, stromal and smooth muscle cells	Glandular epithelium, stromal and smooth muscle cells	63, 112
Placenta	Labyrinth		+		99–101
Testis	Leydig cells	Primary spermatocytes	Endothelial cells of interstitium	Endothelial cells of interstitium	130, 194, 238
Prostate	Prostate cancer		+		127, 179
Heart	Cardiovascular tissue, cardiac cells		Cardiovascular tissue, cardiac cells		229, 230
Kidney	Epithelial tubules		+	Endothelial cells	129, 151
Adrenal gland	Glomerulosa, fasciculate cells	Glomerulosa, fasciculate and endothelial cells	Glomerulosa, fasciculate and endothelial cells	Glomerulosa and endothelial cells	127, 138, 152
Liver		Kupffer cells			151, 160
Pancreas	Pancreatic islet, stellate cells		Vascular endothelial cells	Vascular endothelial cells	138, 139
Stomach	Stomach fundus		Stomach		136, 151
Intestine	Mucosa and mesenchyme of embryonic gut	Enteric plexus	Enteric neuronal crest cells; epithelial cell, ileum submucosal and myoenteric neurons, colon myoenteric nucleus	Ileocecum enteric plexus	175
Adipocytes	+		Preadipocytes		151, 221
Bone marrow and blood cells	B and T cells	Hematopoietic stem cells, monocytes, neutrophils, dendritic cells. Inflammatory granulocytes and macrophages	Hematopoietic stem cells, mouse granulocytes	Hematopoietic stem cells, human and mouse granulocytes	131, 160

expressed in the mucosa and mesenchyme of the mouse embryonic gut. On the basis of in vitro studies, it has been proposed that EG-VEGF/PROK1 might modulate both proliferation and differentiation of enteric neural crest cells (NCC) during enteric nervous system development, which eventually contribute to the formation of the myenteric and submucosal enteric plexus of the bowel rich in PKR1 (172, 174). Both EG-VEGF/PROK1 and Bv8/PROK2 are expressed in mouse and human myocardial tissue where, through PKR1 signaling promotes cardiomyocyte survival and angiogenesis and via Akt activation, protect cardiomyocytes against oxidative stress (230). Recently, studies aimed toward developing cardioprotective agents reported that some non-peptide prokineticin agonists promote angiogenesis and protect heart function in a mouse model of myocardial infarction (82, 231). The clinical relevance of these encouraging findings remains to be established, considering the challenges in the clinical translation of data in animal models of ischemic disease (60).

Bv8/PROK2, but not EG-VEGF/PROK1, is expressed in normal liver, but only in Kupffer cells, the liver resident macrophages (161). Bv8/PROK2 is expressed in the skin, granulocytes, dendritic cells, and macrophages, and its expression increases following local inflammation (84, 131). In rats and mice, inflammatory granulocytes and macrophages strongly express Bv8/PROK2 in the two isoforms, PROK2 and PROK2L (150). By two-dimensional electrophoresis and biochemical purification, Giannini et al. (84) identified and isolated from rat peritoneal granulocytes a peptide, which displayed comparable receptor affinity and biological activity as that of the amphibian Bv8. Also, Zhong et al. (250) purified human Bv8/PROK2 from human neutrophils, tested it for the biological activity in NFAT-CHO cells transfected with *PKR1* cDNA, and also demonstrated its ability to promote neutrophil migration at very low concentrations, suggesting that the Bv8/PROK2 may physiologically regulate neutrophil migration via ERK and phosphatidylinositol 3-kinase (PI3K) pathways.

It noteworthy that the expression levels of the prokineticins, as well as those of their receptors, may be differently regulated in pathological states (86, 179, 224). A whole genome analysis of genes differentially regulated in human aorta following rupture of abdominal aneurysm revealed that Bv8/PROK2 was significantly upregulated, similar to IL-6 and IL-8, suggesting that Bv8/PROK2 is a participant in the immune and inflammatory response associated with such pathological processes (38).

Prokineticins were detected in CNS by Melchiorri et al. as early as 2001 (155). By in situ hybridization and immunohistochemistry using a polyclonal antibody raised against an epitope common to both forms of mouse Bv8, they detected the presence of Bv8-like proteins in layer II of the cerebral cortex, in limbic regions, in cerebellar Purkinje cells, and in dorsal and ventral horns of the spinal cord. Subsequent studies demonstrated some species differences in CNS prokineticin distribution (35, 164, 167).

Maftei et al. (143) reported the presence of Bv8/PROK2 in mice spinal cord mainly in astrocytes. Bv8/PROK2 is also present in peripheral nervous system in some dorsal root ganglion (DRG) neurons, which also express the vanilloid receptors TRPV1 (104, 165). But after injury of the sciatic nerve, immunoreactivity for Bv8/PROK2 becomes evident in many DRG neurons, in calcitonin gene-related peptide (CGRP)-positive nerve fibers and in Schwann cells along the fibers.

Zhou and co-workers reported a detailed description of prokineticin and of prokineticin receptor expression in the CNS of adult mice (37, 248) and of monkey (24). The expression patterns of Bv8/PROK2 and PKR2 in the macaque brain were found to be quite similar to those in the mouse brain. Moreover, through a transgenic reporter approach, they were able to obtain a map of the efferent projections of Bv8/PROK2-expressing neurons in the suprachiasmatic nucleus (SCN). PROK2 might be axonally transported and released at the terminals, but it also acts in a paracrine and/or autocrine fashion. The rhythmic oscillation of Bv8/PROK2 mRNA in the SCN and the widespread expression of PKR2 in major SCN targets support a role of Bv8/PROK2 in circadian-controlled processes. PROK2 oscillation in the SCN is dependent on the core SCN oscillators as it is abolished in mutant mice lacking the functional clockwork (34, 35).

IV. PROKINETICIN RECEPTORS

A. Structure

Three groups independently identified two closely related GPCRs for Bv8/EG-VEGF/PROKs (138, 151, 211), which showed ~80% identity to the previously described mouse orphan receptor gpr73 (178). These receptors are now called prokineticin receptor 1 (PKR1) and prokineticin receptor 2 (PKR2). **FIGURE 4** shows a schematic structure of human PKRs. PKR1 and PKR2 are similar to the neuropeptide Y (NPY) receptor and belong to the family-A of GPCR with two Cys residues located on the first and second extracellular loop giving rice to a disulfide bond. In humans, the genes encoding these receptors are on two different chromosomes (*PKR1* gene: 2p13.3; *PKR2* gene: 20p13), but the sequences of both receptors are highly conserved, with nearly 85% identity; in the mouse, the genes are located on chromosome 6 and 2, respectively. Most sequence variation is concentrated in the extracellular NH_2-terminal region, which contains a nine-residue insert in hPKR1 compared with hPKR2, as well as in the second intracellular loop and in the COOH-terminal tail. Both receptors are encoded by

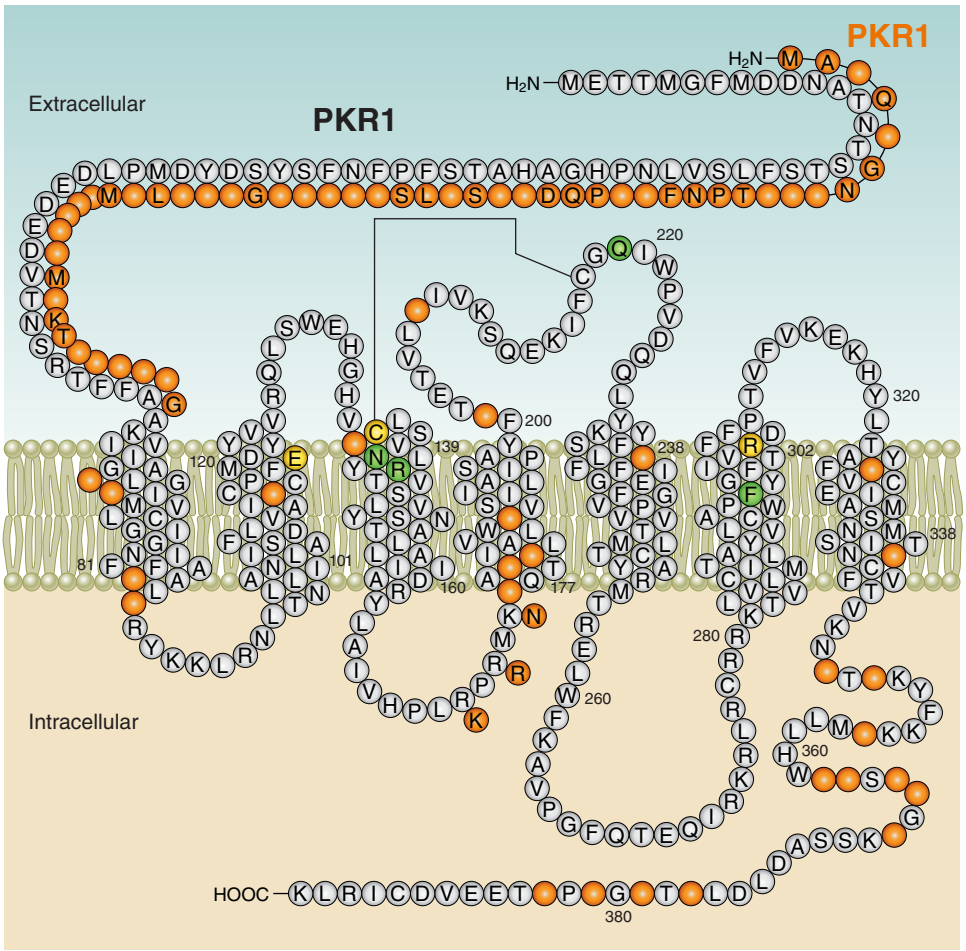


FIGURE 4. Schematic structure of human PKRs. PKR1 (black) has 85% homology with PKR2. Most sequence variations are concentrated in the extracellular NH_2-terminal region, nine residues shorter in hPKR2 (red), as well as in the second intracellular loop [2 Arg residues in PKR1 instead of Asp and Lys in PKR2 (138)] and in the COOH-terminal tail. They belong to the family-A of GPCR with two Cys residues located on the first and second extracellular loop giving rise to a disulfide bond. The binding sites for the endogenous peptide ligands are in correspondence to the second extracellular loop (EL-II); those for the small non-peptide PKR antagonists and agonists are in a different area of an allosteric transmembrane pocket: yellow circles indicate the residues interacting with antagonist, and green circles indicate the residues interacting with agonists. The PKRs are G_q-coupled receptors but may also couple G_i and G_s proteins, indicating that multiple pathways are involved in prokineticin signaling with both tissue and species-to-species variation. [Modified from Maldonado-Pérez et al. (144).]

two exons separated by an intron located at the border of transmembrane domain III (138).

Except for MIT-1, a clearly PKR2-preferring ligand, and PK2β (the predicted short form of PROK2) which displays clear selectivity for PKR1, all the other natural prokineticins bind and activate both receptors in nanomolar range, with Bv8/PROK2 showing a moderately higher affinity than PROK1 for both receptors (138, 211). The non-mammalian prokineticins (MIT-1 and Bv8) display considerably higher affinity with at least one order of magnitude higher compared with human prokineticins (170). Bv8, which displays similar affinity for both receptors, behaves as mammalian PROK2 and is a good pharmacological tool to evaluate the prokineticin activities (168).

Computational (132) and genetic (159) analyses indicate that the binding sites for the endogenous peptide ligands reside on the extracellular surface of the receptors in correspondence of the second extracellular loop (EL-II), whereas small non-peptide agonists and recently identified antagonists (9, 82) bind to an allosteric transmembrane site. However, small agonists and antagonists interact with different residues (Arg144, Asn 141, Gln219, Phe 300 the agonist; Arg 144, Arg307, Cys137, Glu119 the antagonist) resulting in a totally different area of this pocket. This might explain why the available antagonists show only preferential affinity for PKR1 with respect to PKR2, whereas the agonists behave as positive allosteric modulators selective for PKR1 (82).

It has been reported that the signal transduction efficiency of PKR1 may be higher than that of PKR2 (127, 138, 180), suggesting that the net amount of positive charges at the central portion of the second intracellular loop (IL-II), higher in PKR1 (because of the presence of the strong positive-charged amino acid Arg) than in PKR2 (containing the neutral amino acid Asp and the moderate positive-charged Lys), explains a full signaling efficacy and potency.

Receptor internalization (endocytosis) is a mechanism adopted by most receptors to prevent uncontrolled stimulation, which may otherwise lead to disregulation and disease. In vitro studies [polyoma middle T-transformed mouse coronary endothelial cells H5V (81) expressing PKR1, CHO transfected with PKR1, HEK-293 tranfected with PKR2] indicated that both PKRs undergo rapid (in 1 min) agonist-induced internalization which is maximal at 30–60 min and the receptor recycles back to the plasma membrane after the removal of ligand (82, 91, 180). Ac-

cordingly, Mollay et al. (158) observed strong tachyphylaxis to Bv8-induced guinea pig ileum contraction, which developed after the first exposure to Bv8 and lasted for more than 1 h. Also, Cheng et al. (35) reported that persistent intracerebroventricular infusion of Bv8/PROK2 may desensitize the receptors in vivo. Using enhanced green fluorescent protein (EGFP)-tagged PKR2 expressed in HEK-293 cells, Yin et al. (244) found that PROK2-induced PKR2 endocytosis is GRK2- and clathrin-dependent, but β-arrestin-independent.

GPCR activity may be modulated also by accessory proteins, single-pass transmembrane proteins that regulate trafficking and/or signaling of the receptors to which they bind. Recently, Chaly et al. (31) hypothesized that PKR1 might interact with MRAP2, one of the melanocortin receptor accessory proteins, at least in neurons of arcuate nucleus where they colocalize. In transfected CHO cells and in hypothalamic neuronal GT1-1 cell line, they demonstrated that MRAP2 interacts with both PKR1 and PKR2 but significantly and specifically inhibits PKR1 signaling, probably contributing to energy homeostasis regulation (see sect. VC). Accordingly, Mrap2-KO mice were hypersensitive to PKR1 stimulation.

Marsango et al. (148) reported that PKR2 has a dimeric structure in human neutrophils and, by using heterologous expression in *Saccharomyces*, suggested that dimerization proceeds through interactions between transmembrane domains TMs 4 and 5, with a role for TM5 in modulation of PKR2 function (213). They also observed PKR1/PKR2 heterodimers.

Discovery that several *PKR2* mutations are associated with congenital diseases (hypopituitarism, KS, Hirschsprung's disease) points out that discordant effects of various *PKR2* mutations may indicate domain-specific effects and stimulated studies to identify the critical structural elements of the receptor. Functional studies of the mutated receptors transfected in cell lines showed that point mutation of basic amino acids in intracellular loop I or II or III may result in reduction of normal cell surface expression and ligand binding capacity, reduction of receptor interaction with $G\alpha_q$ and $G\alpha_i$, and of receptor endocytosis (2, 180, 254). In the COOH terminus (amino acids 333–384) of PKR2, interactive motives for Snapin, one of constituents of SNARE complex, were mapped (212), implying a role of Snapin in the trafficking of PKR2.

B. Signal Transduction

The PKRs are G_q-coupled receptors and promote intracellular Ca^{2+} mobilization (138, 151, 170, 211, 232) via activation of phospholipase C (PLC)-β and formation of IP_3. PLC inhibition prevents the effects of Bv8 on chemotaxis of mouse macrophages which express mainly PKR1 (150). In contrast, Bv8/PROK2-induced ERK phosphorylation and chemotaxis of human monocytes, mainly expressing PKR2, are inhibited by pertussis toxin (131), and EG-VEGF/PROK1-mediated CXCL8 monocyte production is sensitive to pertussis toxin (160), suggesting involvement of the G_i proteins. Possible homo- or heterodimerization, demonstrated in human neutrophils (148), can modulate signaling by negative or positive binding cooperativity (214). PKRs may also couple G_s proteins (32).

In the DRG, PKR activation, via G_q, increases intracellular calcium and induces protein kinase C (PKC)-ε translocation to plasma membrane (232). In cultured cortical neurons and cerebellar granule cells, Bv8-stimulated mitogen-activated protein kinase (MAPK)/PI3K mediates neuroprotection and cell survival (155). PROK1/PKR1 signal activates the calcineurin/nuclear factor of activated T cells (NFAT) pathway in human endometrium via $G_{q/11}$-ERK to regulate gene transcription and in epithelial cell line induces inositol-phosphate mobilization with sequential phosphorylation of c-Src, epidermal growth factor receptor-MAPK-ERK pathway (47). According to Guilini et al. (91), PKR1 expressed in endothelial cells of arterioles and vessels signals through G_{q11} cells and induces MAPK and PI3/Akt phosphorylation, promoting angiogenesis. In contrast, PKR2 is coupled to $G\alpha_{12}$ in endothelial cells, in which it internalizes $G\alpha_{12}$ and downregulates ZO-1 expression, leading to vacuole formation and the fenestration of these cells. On the basis of transgenic overexpression in the mouse heart, it has been proposed that PKR1 mediates angiogenesis and cardiomyocyte survival (230), while PKR2 would mediate increased vascular permeability (229).

C. Expression

Although both PKR1 and PKR2 are coexpressed in various tissues including brain, PKR1 is mainly expressed in peripheral tissues, including endocrine glands and organs of the reproductive system, the GI tract, spleen, pancreas, lungs, heart, and blood cells (12, 211). In the CNS, PKR2 is more abundantly expressed than PKR1 in several discrete brain regions (35, 138).

It has been reported that PKR1 and PKR2 are differentially expressed in the vascular endothelium in distinct organs (91, 115, 161). In the mouse testis, *pkr1* and *pkr2* are equally expressed, while in the human testis *PKR1* has higher expression than *PKR2*. PKR1 and Bv8/PROK2 are expressed in mouse and human myocardial tissues and might be functional in the heart. Downregulation of Bv8/PROK2 and PKR1 was reported in explanted hearts from patients with end-stage heart failure (reviewed in Ref. 162).

Expression of PKRs by hematopoietic cells is species-specific: unlike mouse neutrophils, which express both PKR1- and PKR2-mRNA (150), isolated human neutrophils ex-

press only PKR2, which is increased in G-CSF-treated donors and in granulocyte-macrophage colony stimulating factor (GM-CSF)-treated human neutrophils and bone marrow in vitro. Both receptors are expressed, at protein levels, on the surface of human monocytes (250).

In the gut, PKR1 is more abundantly expressed than PKR2. An aboral increase in PKR1 expression along the length of rat intestine has been noted: PKR1 immunoreactivity was visualized in epithelial cells, in submucosal and myoenteric neurons of ileum, and in myenteric neurons of mouse colon colocalized with a small subset of neuronal nitric oxide synthase expressing neurons. This receptor localization explains the effects of EG-VEGF/PROK1 on propulsive motility and GI secretions (234).

In the nervous system, both prokineticin receptors can be detected as early as embryonic day 7 in mouse and rat (168). It has been reported that, in cultures of neurons, astrocytes, and microglia isolated from embryonic mouse cerebrum, PKR2 is prevalently expressed in the neurons. In contrast, PKR1 is mainly expressed in astrocytes and microglia (119, 201). PKR1 (but not PKR2) is present in enteric NCCs, where it may be upregulated by GDNF and potentially provide a complementary pathway to glial-derived neurotrophic factor (GDNF) signaling to generate neurons and glia of the enteric nervous system during fetal development (175). It has been also hypothesized that insufficient or improper PROK1/PKR1 signaling may contribute to cause absence of enteric ganglia, as seen in Hirschsprung's disease in humans, or to improper differentiation of NCCs in adrenal glands and sympathetic nervous system to give rise to neuroblastoma, the most common type of childhood solid tumor (125).

Negri et al. (168) reported that in rat embryos, from day 12 both prokineticin receptors are highly expressed in the neuroepithelium lining ventricles, olfactory bulb, Gasser-ganglion, and DRG. One day after birth, PKR2 is still expressed at high levels in multiple areas, whereas PKR1 expression is detected only in the cortex. In adult rats, only PKR2 is appreciably expressed in several brain areas (168).

V. PHYSIOLOGICAL AND PHARMACOLOGICAL EFFECTS OF PROKINETICINS

A. Neurogenesis

In mammals, neurogenesis occurs primarily during embryonic and early postnatal stages, although it persists in certain regions of adult brain (19). Prokineticins function as survival/mitogenic factors for both CNS and peripheral nervous system (PNS). Indeed, in vitro Bv8/PROK2 mediates protection or survival of cortical neurons (35, 155), and PROK1 stimulates proliferation of neuronal precursor cells in enteric nervous system (ENS) (172).

1. Olfatory bulb

PROK2/PKR2 signaling has a critical role in the development of the olfactory bulb (OB). Bv8/PROK2, present in the ependymal and subependymal layers of the olfactory ventricle, is secreted in the OB and functions as a chemoattractant for neuronal progenitors, derived from the subventricular zone (SVZ) and expressing PKRs (120). Genetic analysis in mice indicates that PKR2, but not PKR1, is a critical receptor for OB development. Indeed, all PKR2 knockout mice (but not PKR1 knockout mice) have a dramatic decrease in size and abnormal architecture of the OB receptor-deficient mice (152). Conversely, only 50% of *bv8/prok2* null mice exhibit asymmetric OB morphogenesis (183), suggesting a potential redundancy between the two ligands, EG-VEGF/PROK1 and Bv8/PROK2, in the neurogenesis of the OB. In both *bv8/prok2* and *pkr2* null mice, disrupted gonadotropin-releasing hormone (GnRH) neuron migration resulted in a dramatic decrease in GnRH neurons in the hypothalamus and in the median preoptic area as well as in hypogonadotropic hypogonadism (152, 183). The pathological changes observed in *pkr2* null mice bear a striking resemblance to the clinical manifestations of KS, a human developmental disease with combined features of hypogonadotrophic hypogonadism and anosmia (see next).

2. Enteric nervous system

During embryonic gut development, EG-VEGF/PROK1 is expressed in the mucosa and mesenchyme of the embryonic gut. In vitro, it promotes the survival/proliferation and differentiation of enteric NCCs (172). Recently Ruiz-Ferrer et al. (193) suggested that PKR1 and PKR2 might play a complementary role to the RET/ GFRa1/GDNF signaling pathway, supporting proliferation/survival and differentiation of precursor cells during ENS development. They also identified sequence variants in *PKR1*, *EG-VEGF/PROK1* and *PKR2* genes, which are associated with Hirschsprung's disease, in some cases in combination with *RET* or *GDNF* mutations (193).

B. Circadian Rhythm Regulation

In mammals, the endogenous pacemaker that drives circadian rhythms, such as activity and rest, resides in the SCNs (96). To date, two diffusible molecules, vasopressin and Bv8/PROK2, have been identified as potential candidate SCN output molecules (134).

Bv8/PROK2 mRNA expression levels display a high amplitude of circadian oscillation in the SCN of mice (35) and rats (167), but also of a diurnal rodent (*Arvicanthis niloti-*

cus) and of the monkey, suggesting that the key to diurnality lies downstream of the SCN circadian clock (122). The rhythm of Bv8/PROK2 is directly regulated by the transcriptional/translational feedback loop of the central clock (35). Four E-boxes have been described in both human and mouse Bv8/PROK2 promoters. In vitro experiments revealed that bv8/prok2 transcription is regulated by clockwork gene products through the activation of the E-boxes. These findings were validated in vivo: *bv8/prok2* mRNA expression in the SCN was absent or substantially reduced in mutant mice lacking a functional clockwork, including Clock mutant mice and in Cry1−/−Cry2−/− mice (34, 35).

Also, it has been reported that light can directly regulate Bv8/PROK2 expression in the SCN, provided that the melanopsin and rod-cone photoreceptive systems are intact (34, 35, 251, 252). Neurons expressing *Bv8/PROK2* mRNA are scattered in the dorsomedial SCN, which receives direct projections from the retinal ganglion neurons, thus explaining this photoresponsive regulation. Some of these neurons also express vasopressin (~50%), and the amplitude of the circadian oscillation of Bv8/PROK2-mRNA in the SCN has been shown to be attenuated in the SCN of V1a-deficient mice (135). PKR2 is expressed in essentially all the primary SCN target sites, including the lateral septum (LS), paraventricular talamic nucleus (PVT), paraventricular nucleus (PVN), and dorsomedial hypothalamic nucleus (DMH) (35, 37). Bv8/PROK2 was shown to regulate the excitability of PKR2-positive neurons, such as the neurons of a primary SCN target, the PVN (245), or the SCN neurons themselves (25, 189). The molecular mechanism responsible for increasing the firing rate of SCN neurons depends on a cooperation with the vanilloid channel TRPV2 coexpressed with PKR2 in the SCN neurons (25).

Intracranial delivery of Bv8/PROK2 into the lateral ventricle during subjective night, when endogenous Bv8/PROK2 is low, inhibited the nocturnal wheel running activity of rats (35). Genetic deletion of *bv8/prok2* or its receptor *pkr2* in mice leads to almost identical defects in circadian rhythms (133, 184). In the absence of Bv8/PROK2 signaling, the amplitudes of circadian locomotor parameters were remarkably reduced, with rhythmicity amplitude of wheel-running activity of Bv8/PROK2-deficient or PKR2-deficient mice <20% of wild-type mice (133, 184). Rhythmicity of other circadian parameters, including sleep/wake cycle, body temperature, circulating glucocorticoid and glucose levels, as well as the expression of peripheral clock genes, was also significantly reduced (133, 134, 184, 253).

It has been speculated that patients with mutations in these genes may have circadian rhythm alterations. However, sleep and mood disorders or abnormalities in other circadian phase markers (melatonin, cortisol, and core body temperature) have not been unambiguously demonstrated in human *PKR2*-mutation carriers, indicating an intact central circadian pacemaker activity in these patients, indicating a discordance in the regulation of circadian phenotypes between humans and mice (7, 114).

C. Ingestive Behavior and Energy Homeostasis

The hypothalamus is important for the regulation of energy homeostasis, and PKRs are expressed in hypothalamic nuclei known to regulate ingestive behavior. Inputs to the arcuate nucleus (ARC) come from the SCN, medial preoptic area (MPA), and nucleus tractus solitarius (NTS) whose neurons contain Bv8/PROK2. In rodents, intrabrain administration of the amphibian or mammalian Bv8/PROK2 causes anorexogenic (80, 167) and dipsogenic effects (167); stimulates the release of vasopressin, oxytocin, and corticosterone; and increases the blood glucose level (253). The anorexic effects of Bv8/PROK2 depend on specific activation of PKR2 on ARC neurons releasing α-melanocyte stimulating hormone (80, 167). Intrabrain injection of Bv8 potently stimulated drinking (167) through a direct activation of PKR2 present in the subfornical organ, as demonstrated soon afterwards in vitro (49, 74).

Bv8/PROK2 displays anorectic effects also by peripheral administration. In this case, its effect is mediated via the brain stem and requires PKR1 but not PKR2 signaling; indeed, anorexic effects of PROK2 are completely absent in PKR1-deficient mice (13). PKR1 also participate in regulation of energy homeostasis, being regulated by melanocortin receptor accessory protein 2 (MRAP2) (31). Chronic administration of Bv8/PROK2 reduced food intake and body weight in a mouse model of obesity, without inducing tachyphylaxis, as other anorectic agents do. These findings raise the possibility that PKR1-selective agonists might have therapeutic potential for the treatment of obesity (13). Moreover, PKR1, expressed by preadipocytes and adipocytes (211), may have a role in controlling adipose tissue mass by limiting proliferation and conversion of preadipocytes to adipocytes. Interestingly, late age PKR1-deficient mice have been reported to have peripheral obesity with a diabetes-like syndrome (221).

Both Bv8/PROK2 and PKR1 are sensitive to the nutritional status: food deprivation reduces their expression in rat hypothalamus (80), but Bv8/PROK2 expression was rapidly induced in the mouse hypothalamic PVN after fasting (253). This sensitivity has already been established by the early neonatal period in male and female rats, suggesting that Bv8/PROK2 may compensate for the immaturity of other appetite-regulatory systems during the early neonatal period (107).

PKR1 signaling in endothelial cells has been reported to promote insulin uptake, in vitro and in vivo, in addition to regulating endothelial cell proliferation (57). According

to these studies, impaired capillary formation, transendothelial insulin uptake, and insulin signaling in endothelial-specific PKR1-deficient mice lead to cardiomyopathy, renal disorders, and lypodystrophy. High plasma free fatty acid levels and low leptin levels further contribute to the development of insulin resistance at the later age. Hence, it has been speculated that targeting endothelial PKR1 may serve as a therapeutic strategy for ameliorating these disorders (57).

High levels of prokineticins have been found in obese adipose tissues (233). Bv8/PROK2 is correlated with various cardiometabolic risk factors including blood lipid, blood glucose, blood pressure, BMI, and uric acid. Furthermore, high levels of blood Bv8/PROK2 are independently associated with metabolic syndrome in a Chinese population. Nevertheless, whether there is a causal relationship between Bv8/PROK2 and metabolic syndrome remains to be established (236).

D. Mood Regulation

Given that the expression of Bv8/PROK2 in the SCN is modulated by clock genes and that PKR2 is expressed in SCN targets involved in the mood regulation (such as the amygdala, the lateral septum, and the paraventricular nucleus), studies have been performed in mice to determine whether Bv8/PROK2 may serve as molecular connections of circadian rhythm and mood disorders. Hu et al. (103) reported that *bv8/prok2* null mice had disruptions in the homeostatic regulation of sleep. Li et al. (135) reported that intracerebroventricular infusion of Bv8/PROK2 increased anxiety-like behavior and also led to increased depression-like behavior. Conversely, mice lacking the *bv8/prok2* gene displayed significantly reduced anxiety and depression-like behaviors. Lattanzi et al. (manuscript in preparation) evaluated anxiety-related behavior in mice lacking the *pkr1* gene and in mice treated with a nonpeptide antagonist of the receptor1 and found, in both cases, lower anxiety-related behavior than in wild-type mice.

Also in humans, disrupted circadian rhythms have been associated with several mood disorders, including bipolar disorders (BP) and major depressive disorder (MDD) (145). However, the only study which suggests a possible association between *PKR2* gene mutations and MDD and BP (114) is very small in number and power and so far has not been replicated.

VI. ROLE OF PROKINETICINS IN PATHOLOGICAL CONDITIONS

A. Nociceptive and Chronic Pain

The first evidence of the hyperalgesic effects of the prokineticins came from the observation that systemic administration of the amphibian Bv8 protein induced a characteristic biphasic hyperalgesia to tactile and thermal stimuli in rats (170). The initial phase of hyperalgesia is caused by a local action on nociceptors; the secondary phase of hyperalgesia is attributable to a central action (52, 165, 169), indicating differences in the contribution of PROK/PKRs at central versus peripheral sites. The amphibian protein Bv8 was a useful research tool to characterize prokineticin pharmacology. It induces hyperalgesia by activating the PKRs that are expressed in regions of the nervous system associated with pain. At peripheral levels, within DRG, PKR1 is mainly expressed on small nociceptors together with the transient potential receptor vanilloid 1 (TRPV1), and PKR2 is expressed on medium/large-sized neurons expressing TRPA1 besides TRPV1. These colocalizations provide the anatomical basis for a cooperative interaction in nociceptor sensitization through activation of PKC-ε (232). But other additive processes may lead to a cumulative sensitization. In rat primary sensory neurons, PROK2 also enhances proton-gated current, suppresses GABA-activated current, and sensitizes P2X receptors, via PKC signaling pathway (185, 188, 241). Half of the Bv8/PROK2-responding DRG neurons co-expressed neuromediators implicated in pain processing, including CGRP and substance P and release these neuropeptides upon exposure to Bv8 (52). Some PKR1-positive neurons also express PROK2. Evidence that the PROK2/PKR system is directly involved in setting the pain threshold comes from studies in mice lacking *pkr1* or *pkr2* or *prok2*: all these genotypes display higher thermal, mechanical, and tactile pain threshold than wild-type (WT) mice (104, 165). Exposure of DRG cultures to GDNF induced expression of functional PKRs (232) in agreement with the demonstrated upregulation of PKRs after tissue damage and inflammation (143). In skin preparations, Bv8 sensitizes the peripheral nerve endings to heat, lowering threshold and increasing heat response through TRPV1 activation (102).

Intrathecal administration of Bv8 (in the range of fmol) induces biphasic hyperalgesia, and intrathecal administration of PKR antgonists rapidly and dose-dependently reduces the hyperalgesia induced by inflammation or tissue damage, i.e., when the PROK system is activated (124, 143).

The PROK2/PKR system may also intervene in modulating central pain mechanisms (53). This proceeds from inhibiting the endogenous PAG-RVM antinociceptive pathway (53, 124) and decreasing the encephalinergic tone in the area postrema, a sensory circumventricular organ in the medulla that lacks a blood-brain barrier, and is rich in PKR2. Indeed, Ingves and Ferguson (105) reported that Bv8/PROK2 causes membrane depolarization and suppression of action potential firing in the majority of ENK-expressing neurons of the AP, suggesting that Bv8/PROK2 has the ability to suppress opioid release from these populations of cells.

B. Inflammatory Pain

Tissue injury and inflammation result in release of various mediators that promote ongoing pain or pain hypersensitivity against mechanical, thermal, and chemical stimuli. Bv8/PROK2, overexpressed in human and animal inflamed tissues predominantly in infiltrating neutrophils (84, 186, 207), is one of the main pronociceptive mediators that activates primary afferent neurons to enhance nociceptive signal transmission to the CNS and is also an important modulator of immune responses. In an animal model of inflammation produced by complete Freund's adjuvant (CFA) injection into the paw of rats or mice, Bv8/PROK2 mRNA, which is quite undetectable in healthy paw, dramatically increases in the skin, associated with infiltrating cells (granulocytes and macrophages) and temporally correlates with pain and other traits of inflammation as edema, but 24 h after the injection, it is significantly increased also in the DRG ipsilateral to the paw injected with CFA (84, 163). Granulocyte-released Bv8/PROK2 (see also sect. VIG) modulates acute inflammatory pain directly acting on nociceptors and, in turn, exerts chemotactic activities, induces a proinflammatory macrophage phenotype, and skews the Th1/Th2 balance to Th1 (58, 72, 150). Hence, besides the direct activation of the nociceptors by Bv8/PROK2, other cytokines/chemokines, induced by Bv8/PROK2, contribute to keep pain in chronic inflammation. Deletion of the *pkr1* and *pkr2* genes substantially reduced inflammation-induced thermal and mechanical hypersensitivity, but only deletion of the *pkr1* gene reduced the PROK2 upregulation, indicating that while both receptors are responsible for pain, only PKR1 is implicated in mediating the enhanced Bv8/PROK2 expression level during the inflammatory process (84). Peripheral and topical administration of PKR antagonists, A-24, PC1 or PC7, significantly reduced pain, but also edema and extravasation, consistent with the effect of Bv8/PROK2 on vascular permeability (84, 124, 169).

Prokineticin system dysregulation has been reported in a mouse model of human rheumatoid arthritis [type II collagen-induced arthritis (CIA)]. Bv8/PROK2 is dramatically upregulated in the thickened synovial membrane of arthritic mice with a time course that parallels the arthritis score (121). Chronic treatment with prokineticin receptor antagonists significantly reduces the thermal hypersensitivity and the histological damage, encouraging a possible application of Bv8/PROK2 inhibitors to combat arthritic inflammation and pain (106).

C. Neuropathic Pain

Neuropathic pain, resulting from damage to or dysfunction of the nervous system, is a chronic condition characterized by abnormal pain perception, such as hyperalgesia (increased pain perception of noxious stimuli), allodynia (hypersensitivity to normally innocuous stimuli), and neuronal oversensitization occurring at the spinal level and leading to abnormal pain transmission. It is associated with neuroinflammation-related events that participate in pain generation and chronicization (48, 79).

Peripheral nerve damage [chronic constriction injury (CCI), spared nerve injury (SNI) in mice] induces overexpression of PROK2 and of its receptor PKR2 both in peripheral nerve and in the spinal cord.

PROK2-mRNA upregulation starts in the nerve 3 days after injury and moves towards the center, being significant in the spinal cord 10 days after injury. PROK2 protein becomes detectable in some axons of the damaged nerve but is mainly associated with activated Schwann cells and infiltrating macrophages. The release of Bv8/PROK2 in the nerve contributes to neuroinflammation. Ten days after injury, Bv8/PROK2-mRNA and protein are significantly increased also in the DRG neurons and in spinal cord, in activated astrocytes, but not in microglia, consistent with the demonstration that STAT3, the enhancer of Bv8/PROK2 transcription in myeloid cells (186, 207, 242), is activated by G-CSF, IL-6 and IL-1β signaling in DRG neurons and astrocytes, but not in microglia (199, 228). In the spinal cord, the increased Bv8/PROK2-immunofluorescence associated with synaptophisin (a presynaptic marker) indicates that PROK2 may be transported to the central endings of nociceptors and released. Bv8/PROK2, overexpressed with the PKR2 on activated astrocytes functions as an astrocytic-autocrine-growth factor (119, 201). Eventually Bv8/PROK2 released in the spinal cord activates the PKR2 constitutively localized on the projection neurons and upregulated after nerve injury (143), contributes to spinal glia activation, and results in aberrant excitability in the dorsal horn, with allodynia, the marker of neuropathic pain. It should be pointed out that nerve damage induced PKR2 overexpression in all the examined tissues: nerve, DRG, and spinal cord. Treatment with prokineticin antagonists, such as PC1, was efficacious in controlling and preventing neuropathic pain. This treatment delayed the recurrence of painful symptoms following PC1 suspension, raising the possibility that blockade of Bv8/PROK2-signaling might result in long-lasting changes in the neuronal circuits, or in the neuroinflammatory processes involved. PC1 treatment also normalizes the nerve injury-increased permeability of the blood-spinal cord barrier (BSCB) (90, 143), demonstrating the possible involvement of the PROK system in the regulation of the neuroinflammatory phenomena leading to infiltration of the peripheral immune cells into the spinal cord.

Neuropathic pain is often a consequence of diseases such as diabetes (76). In a mouse model of streptozotocin-induced diabetes, the PROK system was implicated both in the early stage of allodynia development as well as in its maintenance (29). In this animal model, the authors demonstrate that

pharmacological blockade of the system with PC1, besides controlling pain and immune system disregulation, also prevents the upregulation of GluNB2-subunit of the NMDAR, distributed in spinal cord dorsal horns and known to mediate nociceptive hypersensitivity induced by peripheral injury or tissue inflammation (18, 225), suggesting a positive loop between PROK2 and glutamatergic transmission [see also Caioli et al. (26)].

Common cancer types such as prostate, breast, and lung cancer have a tendency to metastasize to bone and induce bone pain (CIBP), which can be seriously disruptive to the patients' quality of life (64). It is a mixed-mechanism pain state that is not entirely similar to inflammatory or neuropathic pain (64); it is difficult to treat so that at least 20–40% of the CIBP is not adequately controlled. In a rat CIBP model developed by injecting tumor cells (Walker 256 carcinoma) in the medullary cavity of rat tibia, mechanical hyperalgesia developed in 6 days and progressively increased together with increased levels of Bv8/PROK2 in the spinal cord. Intrathecal administration of an anti-Bv8/PROK2 antibody significantly attenuated the CIBP behavior as well as upregulation of spinal tumor necrosis factor-α protein expression (94).

D. CNS Autoimmunity and Inflammation

According to recent studies, Bv8/PROK2 is an important immune regulator of CNS autoimmune demyelination and thus might represent a new target for therapy (1). Bv8/PROK2 levels were increased in the blood of patients with relapsing/remitting multiple sclerosis (MS, an autoimmune demyelinating disease of the CNS), while it was undetectable in healthy controls, and transcripts for Bv8/PROK2 were significantly increased in peripheral blood mononuclear cells (1). In mice with experimental autoimmune encephalomyelitis (EAE), an animal model of MS (46), Bv8/PROK2 (mRNA and protein) is highly expressed in spinal cord, in white matter inflammatory infiltrates. As in patients, Bv8/PROK2 was higher in sera of mice with EAE with respect to naive mice, and PROK2-mRNA was highly expressed in lymph nodes and progressively increases during development of the disease (1). In both MS and EAE, myeloid cells are a major component of CNS inflammatory infiltrates, and mononuclear cell numbers increase in the peripheral blood before EAE relapses. Thus mononuclear cells may be important sources of Bv8/PROK2, which is induced by elevations in plasma levels of G-CSF (see also sect. VIG), peaking early during the priming phase. Pharmacological blockade with Bv8/PROK2 antagonists, both in preventive and therapeutic schedule, significantly reduced the diseases score in chronic EAE (MOG) and in relapsing/remitting (PLP) animal models, reduced inflammation and demyelination, and modulated autoimmune response against myelin antigen in lymph node cells reducing the production of interferon-γ and IL-17α, known to play a crucial role in EAE development and progression (88, 215).

As mentioned above, insults such as ischemia, amyloid β deposition, or neuronal degeneration modulate the PROK2 levels in the brain. In a stroke model, produced by occlusion of middle cerebral artery (36), Bv8/PROK2 expression is increased in ischemic cortex and striatum while PKR2 is increased in ischemic cortex. Bv8/PROK2 upregulation occurs mainly in neurons and is dependent on NMDA receptor activation. Exogenous delivery of Bv8/PROK2 (10 pmol icv) post-stroke worsened the infarct volume and increased CD68+ inflammatory cells in the ischemic infarct, whereas blocking the Bv8/PROK2 actions with an antagonist reduced infarct volume and central inflammation and improved behavioral outcome. These results suggested that Bv8/PROK2 may be an insult-inducible endangering factor. In contrast, Landucci et al. (123) recently reported a protective role of Bv8/PROK2 in "in vitro" models of cerebral ischemia and ischemic tolerance. It is interesting to note that Bv8 and PROK2 were used in the nanomolar range (10–100 nM) in all the studies in which they provided protective or prosurvival effects (123, 155, 171, 231). Conversely, the proapoptotic effects of these prokineticins were typically obtained at much lower concentrations (10–100 pM) (36, 201). As suggested by Landucci et al. (123), the harmful effects of picomolar concentrations of Bv8/PROK2 might be mediated via SAP/JNK pathway (36), whereas the ERK/Akt-mediated prosurvival effects require the activation of a significant fraction of receptors achievable with high concentration of Bv8/PROK2. The relative contribution of PKR1 and PKR2 in these mechanisms still needs to be fully elucidated. It is noteworthy that in any pathological situation where the prokineticin system is activate, at least in mice, the early, apparently harmful increase in PROK2 is followed by a delayed increase of PKR2. Hence, the larger availability of ligand/receptor might result in stronger signaling, enough to activate the ERK/Akt protective pathway leading towards pathology resolution. The internalization of PROK2 receptors might induce formation of signalosomes that activates intracellular cytoprotective transduction pathway (244). This may contribute to a fine-tuned balance of the expression levels of PKR1 and PKR2 as suggested by studies on the involvement of the PROK system in heart and kidney physiology and pathophysiology (162).

It has been proposed that Bv8/PROK2 expression, even if low in the nigral system, is induced in nigral dopaminergic neurons during the early stages of degeneration (before the onset of motor deficits) in mouse models of Parkinson's disease and Bv8/PROK2 expression is elevated in the substantia nigra (SN) of Parkinson's patients (87). Based on functional in vitro studies, they suggest that PROK2, upregulated in surviving nigral dopaminergic neurons, which constitutively express PKR2, promotes mitochondrial biogenesis and activates ERK and Akt survival signaling pathways, thereby driving neuroprotection (87).

Recent studies suggest that the prokineticin system may have a role in the amyloid β-induced neuronal damage (26, 201). Bv8/PROK2 levels were significantly elevated in hippocampus and brain cortex in rodent models of Alzheimer's disease as well as in the blood of Alzheimer's patients (Severini, personal communication). **FIGURE 5** shows that tissue damage or neuroinflammation increases Bv8/PROK2 expression in neurons and glial cells in peripheral and central nervous system.

E. Kallmann Syndrome

In 2005, Ng et al. (171) reported the involvement of the PROK2/PKR2 system in OB morphogenesis in the mouse. PROK2 appeared to function as a chemoattractant for subventricular zone-derived neuronal progenitors. Bv8/PROK2 also acted as a detachment signal for chain-migrating progenitors from the rostral migratory stream (171). PROK2-null mice had significant reduction in OB size, loss of normal OB architecture, and accumulation of neuronal progenitors (171). A year later, Matsumoto et al. (152) reported a similar phenotype in mice lacking PKR2. In contrast, mice lacking PKR1 appeared normal (152). Both *bv8/prok2* and *pkr2* null mice showed a drastic reduction in GnRH-expressing cells in the median preoptic area as well as absence of GnRH neural projections in the medium eminence (152, 183). The reduced number of hypothalamic GnRH neurons was associated with failure of GnRH secretion, low plasma levels of luteinizing hormone and follicle stimulating hormone in male mice, and impairment of sexual development and fertility in both male and female mice. Male *bv8/prok2* and *pkr2* null mice exhibited small seminiferous tubules lacking lumens, and absent haploid spermatocytes and spermatids. Similarly, female *bv8/prok2* and *pkr2* null mice exhibited disrupted estrus cycles as a consequence of incomplete follicular development characterized by absence of mature follicles and corpora lutea, but ovarian function could be restored by gonadotropin replacement (183). Moreover, in wild-type testis, PKR2 was abundantly expressed in vascular endothelial cells in interstitial tissue (126), and PKR2-deficient mice display reduced interstitial space accompanied by small and scattered Leydig cells, compared with their wild-type littermates (152), suggesting that PKR2 could be involved in vascular remodeling. In humans, PKR2 signaling does not directly affect Sertoli cell function (227). In the adult mouse, PKR2 is expressed throughout the pituitary structures, especially in the pars nervosa supporting a role for PKR2 signaling in the pituitary.

The anomalies observed in PKR2-null mice are clearly reminiscent of the clinical manifestations of KS, a human disease which is characterized by the association of idiopathic hypogonadotropic hypogonadism (IHH), secondary to GnRH deficiency, and anosmia (or hyposmia) related to OB agenesis (217). Mice with heterozygous gene deletions are

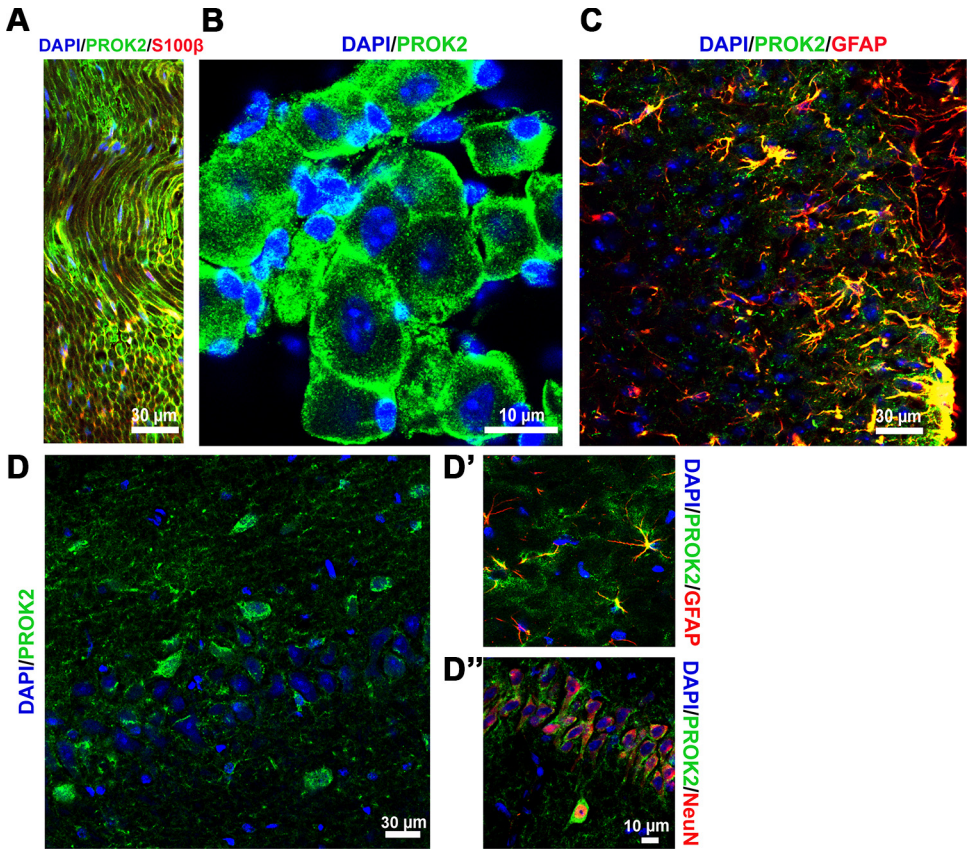


FIGURE 5. Tissue damage or neuroinflammation increases Bv8/PROK2 expression in neurons and glial cells in peripheral and central nervous system. *A–C* are from a neuropathic pain model: chronic constriction injury (CCI) of the sciatic nerve in mice (143). Ten days after injury Bv8/PROK2 immunofluorescence is strongly increased in nerve fibers (green) and in activated Schwann cells (yellow) (*A*), in the body of DRG neurons (*B*), and in activated astrocytes in spinal cord dorsal horns (*C*). *Bottom panels* are from a model of brain neuroinfammation induced by intracerebroventricular influsion of amyloid β (A1-42) in rats. While Bv8/PROK2 immunofluorescence is practically undetectable in the hippocampus of healthy rats (data not shown), 35 days after Ab-infusion, Bv8/PROK2 signal becomes clearly evident in rat hippocampus (*D*). Bv8/PROK2 immunofluorescence is localized in activated astrocytes (*D'*) and in some neurons (*D''*) (unpublished data, courtesy of Dr. Daniela Maftei, Department of Physiology and Pharmacology, Sapienza University of Rome, Rome, Italy).

normal, but the clinical syndromes in humans are predominantly associated with the heterozygous state. Also, humans with identical PROK2/PKR2 mutations show marked variations in both olfactory and reproductive phenotypes and PROK2/PKR2 mutations have been reported also in normosmic GnRH deficiency. Taken together, these findings have led to the recognition of di-oligogenicity in this disease, accounting, at least in part, for the incomplete penetrance of these cases (182, 220).

KS-affected individuals usually do not undergo spontaneous puberty, but GnRH deficiency may be reverted in adults after treatment with sex steroids. In a few patients with PROK2/PKR2 mutation, a persistent oligo/azospermia was observed (209), indicating a primary gonadal defect that is to be correlated with the unique expression profile of Bv8/PROK2 and PKR2 in the testes and, in particular, the expression of Bv8/PROK2 in primary spermatocytes (126, 238), suggesting a role for Bv8/PROK2 signaling in regulating primary testicular function and spermatogenesis.

After the finding that the pathological changes observed in mutant mice were a phenocopy of those observed in KS patients (specifically the arrest of GnRH neuronal migration), the Bv8/PROK2 pathway became an obvious candidate gene to test for the etiology of human GnRH deficiency (56, 195). Until, more than 20 mutations in *PKR2* (see **FIGURE 6**) and more than 10 in *PROK2* have been found in KS patients. Most of these mutations were missense mutations, and many were also found in apparently unaffected individuals. Notably, KS patients harboring biallelic mutations in *PROK2* or *PKR2* have a less variable and more

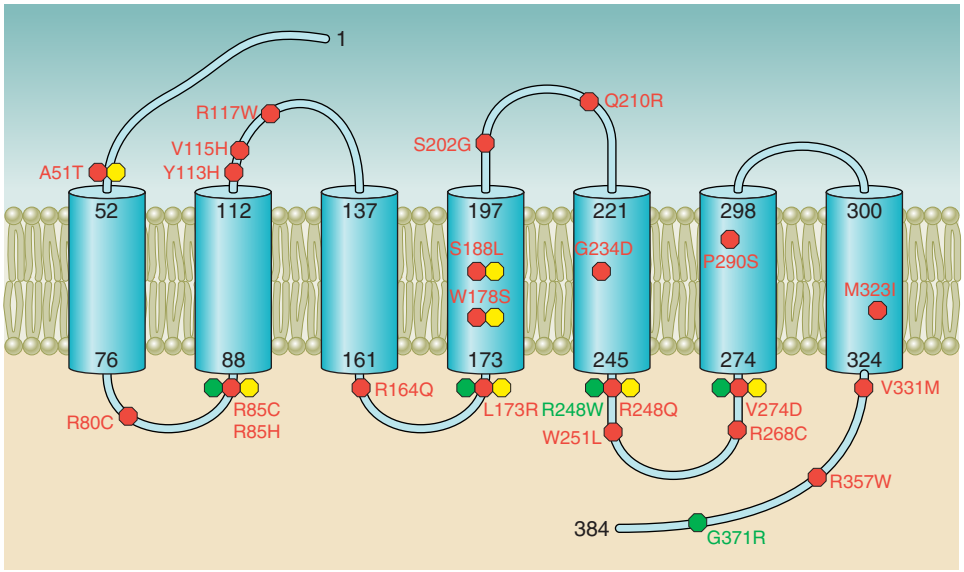


FIGURE 6. Schematic representation of the human PKR2 protein. The 21 missense mutations found in Kallmann syndrome patients are indicated in red. Mutations found in GnRH-deficient normosmic idiopathic hypogonadotropic hypogonadism (nIHH) probands are labeled in green, and mutations found in septo-optic displasia (SOD)/combined pituitary hormone deficiency CPHD/pituitary stalk interruption syndrome (PSIS) patients are labeled in yellow. The mutated PKR2 were functionally assessed in cell lines. Q210R mutation, located in the second extracellular loop (159), interferes with ligand binding and impairs receptor activation. Mutations in the transmembrane domains of the receptor such as L173R, W178S, and P290S impaired cell surface targeting of the receptor. The mutations in the intracellular loops and in the COOH-terminal domain (R85C, R85H, R164Q, R268C, and V331M) interfere with G protein activation. Mutations that strongly impair cell surface targeting of the receptor or binding to Bv8/PROK2 have drastic effects on the different PKR2 signaling pathways, including the major G_q-dependent pathway, and therefore are clearly pathogenic. R164Q is critical for $G\alpha_q$, $G\alpha_i$, and $G\alpha_{16}$ interaction and makes this mutation most likely pathogenic. Arginines in positions 80 and 85 are highly conserved among different species. While R85C and R85H mutations interfered only marginally with receptor function, the R80C mutation was associated with a substantial reduction in receptor activity. When in vitro cotransfected with wild-type, most of the mutant receptors did not affect the signaling activity of the wild-type receptor, arguing against a dominant-negative effect of the mutations in vivo, which is consistent with the lack of phenotypic defects in some heterozygous carriers (153). Mutations with no apparent functional defects (R357W) or with relatively mild defects (A51T, R85C, R85H, M323I, V331M) are not pathogenic. The mutation R268C, frequent in the African-American population (~8% of this population), also in the homozygous state is now considered nonpathogenic. It is tempting to speculate that the PROKR2 R268C variant has undergone positive selection pressure in Africans because the loss of $G_{i/o}$ coupling of this mutant receptor (without G_q coupling impairment) would be advantageous from the evolutionary viewpoint, perhaps by protecting the individuals against infectious agents that would use or target this particular signaling pathway in the infection process (197). PKR2 has been recently identified as a receptor for *T. cruzi* natural infection (113).

severe reproductive phenotype than patients with monoallelic mutations in these genes. The phenotype of patients carrying monoallelic mutations in these genes is more variable, and likely depends on the additional genetic mutations in other causal genes (55), linked to the neurodevelopmental phenotype of KS transmission. Nonreproductive, nonolfactory clinical anomalies associated with KS seem to be restricted to patients with monoallelic mutations. Severe sleep disorders and abnormal circadian phase markers (circadian glucocorticoid secretion, melatonin and core body temperature) as well as obesity and type II diabetes have been seen in a minority of patient with PROK2/PKR2 mutations, but subsequent studies failed to confirm this link in humans (7, 196).

Earlier review articles emphasize the complexity and the unanswered questions regarding the role of the Bv8/PROK2 pathway in the pathogenesis of KS and in human reproduction (8, 149). For a recent review discussing the genetic heterogeneity of KS, see Boehm et al. (16).

Recent studies indicate overlapping phenotypes/genotypes between KS and congenital hypopituitarism, a rare condition that may be associated with complex midline defects of the forebrain (237), including combined pituitary hormone deficiency (CPHD, diagnosed as a deficiency of at least two pituitary hormones) and septo-optic dysplasia (SOD, a disorder characterized by pituitary hormone deficiencies, optic nerve hypoplasia, and midline defects) (237). It should be noted that *PKR2* but not *Bv8/PROK2* mutations were described in patients with CPHD, including SOD (6, 153, 187) and pituitary stalk interruption syndrome (92, 190). These reports suggest a potential role of the Bv8/PROK2 pathway in early pituitary development, as well as in the development of GnRH neurons. However, the extent to which PKR2 variants contribute to either hypopituitarism or KS-associated phenotypes remains to be established.

F. Other Roles of Prokineticins in the Pathophysiology of Reproduction

As discussed in the previous sections, *PROK2* or *PKR2* inactivation results in deficient gonadotropin secretion and reproductive failure secondary to defective neurogenesis of GnRH neurons.

The prokineticins might also play a role in reproduction through the regulation of angiogenesis (127). While *Bv8/PROK2* mRNA is practically undetectable in the human female reproductive organs and placenta, *EG-VEGF/PROK1* expression is strong in the human ovary and undergoes changes during the ovulatory cycles (69). Interestingly, consistent with the association of EG-VEGF/PROK1 expression with steroidogenic cells, particularly intense expression was detected in "hilus cells" (43), a populations of cells involved in androgen production and thought to be the functional equivalent of Leydig cells (216). Early studies documented the intimate association of hilus cells with blood vessels and nerve fibers (156). In situ hybridization analysis (69) also revealed strong expression of EG-VEGF/PROK1 mRNA in specimens of polycystic ovary syndrome (PCOS), a leading cause of infertility, characterized by the association of hirsutism, obesity, and enlarged polycystic ovaries (217). Other key features of this syndrome are hyperplasia and high vascularity of the ovarian stroma, with excessive production of androgens. In the series examined, EG-VEGF/PROK1 mRNA was strongly expressed in PCOS ovaries, with a pattern complementary to the expression of VEGF mRNA. Interestingly, the site of highest expression of EG-VEGF/PROK1 mRNA was the stroma, and thus it was highly correlated with angiogenesis (69).

According to Fraser et al. (73), *EG-VEGF/PROK1* mRNA expression is localized predominantly to granulosa-derived cells of the corpus luteum. These authors reported that human chorionic gonadotropin stimulated both VEGF and EG-VEGF/PROK1 mRNA in vitro. According to these studies, in the human corpus luteum EG-VEGF/PROK1 mRNA expression is mainly associated with granulosa lutein cells, and its synthesis is highest during the mid- to late luteal phase (73).

Battersby et al. (12) reported that, in the human endometrium, expression of *EG-VEGF/PROK1* mRNA was elevated in the secretory phase of the menstrual cycle relative to the proliferative phase, consistent with a regulation of EG-VEGF/PROK1 mRNA expression by progesterone. Others also reported that, in human endometrium, EG-VEGF/PROK1 shows a dynamic pattern of expression across the menstrual cycle and during pregnancy, suggesting that it plays a role in implantation and early pregnancy (71, 73, 99). In addition, the plasma levels of EG-VEGF/PROK1 were reported to substantially increase during pregnancy, consistent with the hypothesis that the placenta is the major source of this factor (99, 101). The same group also investigated the expression pattern of EG-VEGF/PROK1 and receptors in the mouse placenta throughout gestation and found that *Eg-vegf/prok1* and *vegfa* exhibited different expression patterns and different localizations. While *Eg-vegf/prok1* was primarily localized in the labyrinth, *vegfa* was mainly expressed in glycogen and giant cells (100).

Subsequent studies have provided evidence for a potential role of EG-VEGF/PROK1 in multiple disorders of pregnancy (reviewed in Ref. 3).

Gao et al. (78) reported that EG-VEGF/PROK1 plasma levels are a strong predictor of occurrence and severity of ovarian hyperstimulation syndrome (OHSS) in patients undergoing ovulation induction. OHSS is characterized by massive ovarian enlargement and extensive increases in vas-

cular permeability and can be a life-threatening complication (59). Interestingly, PCOS is a well-known risk factor for OHSS (111).

These data, together with a wealth of studies showing a correlation between plasma levels of EG-VEGF/PROK1 and various pathophysiological conditions in humans as well as provocative in vitro studies showing effects of EG-VEGF/PROK1 on endothelial and other cell types from reproductive organs (3, 4, 20–22, 61–63, 77, 100, 116, 235), suggest important regulatory functions of EG-VEGF/PROK1 in the pathophysiology of the female reproductive tract and pregnancy, with interesting therapeutic implications.

However, one has to acknowledge that progress in this field has been hampered by the lack of suitable mouse or rodent models that recapitulate the expression pattern and thus potentially the functional roles that EG-VEGF/PROK1 plays in humans and in other primates. As already noted, the expression pattern of mouse *eg-vegf/prok1* mRNA is different from that of its human ortholog (129), with mouse *eg-vegf/prok1* being expressed primarily in liver and kidney, with little or no expression in endocrine glands (129). As discussed in section II, the human but not the mouse *EG-VEGF/PROK1* promoter (129) has a consensus site for SF-1, which confers steroidogenic cell-specific expression (177). Indeed, there are some major differences in ovarian physiology between rodents and primates. It is well-established that in humans and other primates, a single follicle is selected from the cohort that enters the follicular cycle (98). This single follicle, referred to as the "dominant follicle," establishes dominance and is able to achieve ovulation, unlike the nonselected follicles. It has been hypothesized that the establishment of a more complex and extensive vascular network confers a growth advantage to the dominant follicle (98, 246). Therefore, it is possible that the expression of *EG-VEGF/PROK1* reflects the greater complexity and requirement for a finer regulation of angiogenesis in the primate ovary (129). Further research is needed to test this hypothesis.

G. Role of Prokineticins in Myeloid Cell Biology and in Tumorigenesis

As already mentioned, early studies showed that both EG-VEGF/PROK1 (127, 139) and Bv8/PROK2 (130) are able to induce endothelial cell proliferation, migration, and angiogenesis, suggesting the possibility that these molecules play a role in tumor angiogenesis. Indeed, several studies have documented *EG-VEGF/PROK1* mRNA and protein expression in human tumors derived from steroidogenic cells in the adrenal cortex, testis, and ovary (11, 97, 128, 194, 249).

The possibility that the prokineticins may have some functions in the bone marrow was suggested by the observation that a well-established angiogenic factor such as VEGF-A is expressed by hematopoietic stem cells (HSC) and has the ability to mobilize as well as promote proliferation and survival of blood-marrow-derived cell types by autocrine and paracrine mechanisms (83). Indeed, in the embryo, common progenitors give rise to hematopoiesis and angiogenesis (191), and VEGF-A inactivation results in defective angiogenesis and blood island development (68).

In 2004, LeCouter et al. (131) reported the expression of Bv8/PROK2 as well as of PKR1 and PKR2 in multiple cell lineages from the bone marrow, while EG-VEGF/PROK1 was undetectable. These studies demonstrated the ability of Bv8/PROK2 to promote differentiation, survival, and mobilization of granulocytic and monocytic lineages and also showed that both EG-VEGF/PROK1 and Bv8/PROK2 are able to induce formation of granulocytic and monocytic colonies in human and mouse HSC (131). This study also reported the expression by in situ hybridization of *Bv8/PROK2* mRNA in neutrophils infiltrating human inflamed tissues (131), providing the first evidence linking expression and function of this molecule to a key cell type of the innate immune system. As indicated in previous sections of this review, it is now well established that neutrophils (and macrophages) upregulate Bv8/PROK2 expression in response

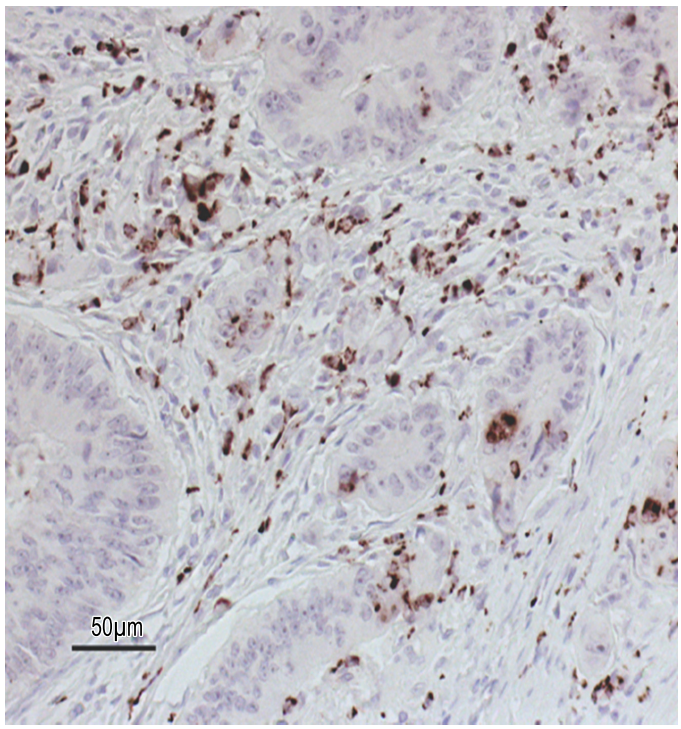


FIGURE 7. Immunohistochemical localization of human Bv8/PROK2 in invasive colon adenocarcinoma. Immunostaining was performed as described (250). Note the strong signal in multifocal, dense neutrophil clusters in the stroma adjacent to invasive tumor, and in neutrophil clusters associated with necrotic debris in tumor gland lumens. (Figure courtesy of Dr. Franklin Peale, Genentech Inc.)

to a variety of proinflammatory stimuli (1, 84, 121, 250). Also, human neutrophils isolated from peripheral blood or bone marrow were reported to strongly express Bv8/PROK2, which in turn stimulated neutrophil chemotaxis (250).

It has been known for a long time that neutrophils and other myeloid cells provide the first line of defense against many pathogens (15). Also, much recent evidence supports the notion that various bone marrow-derived cell types regulate tumor angiogenesis and growth by a variety of mechanisms that are often context-dependent (10, 50, 137, 140). Mantovani et al. (146) reported that macrophages, depending on their "polarization" state, may have either tumor-promoting or tumor-suppressive effects. Recent studies have emphasized the important pathophysiological roles of a population of myeloid cells, defined in the mouse by the CD11b and Gr1 markers (CD11b+Gr1+), which consists primarily of neutrophils, but also includes macrophages and dendritic cells (203). Increased numbers of CD11b+Gr1+ cells (or their functional equivalents in humans) have been reported in tumor-bearing mice and in cancer patients (54, 147, 200, 243). These cells infiltrate tumors and stimulate angiogenesis and tumor growth (39, 208). Subsets of CD11b+Gr1+ cells have been also implicated in suppressing T-cell-mediated immune responses, hence the denomination "myeloid-derived suppressor cells" (MDSC) (75, 147).

In 2007, Shojaei et al. (205) reported that resistance to anti-VEGF antibody treatment in some tumor cell lines implanted in immunodeficient or immunocompetent mice was correlated with tumor infiltration by CD11b+Gr1+ myeloid cells. Subsequent studies reported that Bv8/PROK2 was strongly upregulated in CD11b+Gr1+ cells associated with such resistant tumors (206, 207). Function blocking anti-Bv8 antibodies inhibited tumor angiogenesis and growth and exhibited additive effects with anti-VEGF antibodies in slowing down the growth of anti-VEGF resistant tumors (207).

These studies identified G-CSF as a key inducer of Bv8/PROK2 expression in myeloid cells (207). This induction has been observed in multiple biological contexts, including mononuclear blood cells isolated from human subjects treated with G-CSF (1, 41, 142, 186, 206, 250), and is dependent on STAT3 signaling (186). G-CSF is a key regulator of granulopoiesis and is produced by endothelial cells, fibroblasts, and macrophages in response to various inflammatory cytokines (14, 192). Also, subsets of human tumors produce high amounts of G-CSF, resulting in high neutrophil counts, and, in more severe cases, in "leukemoid reac-

Table 2. *Biologic activities of PROKs and potential associated disorders in mice and humans*

System/Organ	Biological Effects	Associated Pathology in Mice	Associated Diseases in Humans	Factors	Reference Nos.
CNS	Olfactory bulb morphogenesis	Olfactory bulb ipoplasia, olfactory bulb neurogenesis deficiency, GnRH deficiency	Kallmann syndrome, idiopathic hypogonadotropic hypogonadism, impaired development of reproductive axis	PROK2, PKR2	152, 171, 196
	Circadian rhythm regulation	Attenuated circadian rhythms, reduced locomotor activity	Mood disorder?	PKR2	34, 35, 103, 133
PNS	Nociceptive threshold	Altered pain perception	?	PROK2, PKR1, PKR2	102
Gastrointestinal tract	Enteric nervous system development		Hirshprung disease	PROK1, PKR1, PKR2	172, 193
Ovary	Angiogenesis		Polycystic ovary syndrome	PROK1	69, 127, 144
Testis	Angiogenesis		Leydig cell tumors	PROK1	130, 194
Prostate	Angiogenesis		Prostate cancer		179
Endometrium	Angiogenesis		Endometriosis	PROK1	227
Placenta	Trophoblast differentiation		Idiopathic recurrent pregnancy loss	PROK1, PKR1	99–101
Adrenal gland	Angiogenesis		Neuroblastoma progression	PROK1, PKR1, PKR2	127, 138
Bone marrow peripheral blood	Immune responses hematopoiesis	Tissue inflammation/ neuroinflammation	Inflammatory disease, arthritis, multiple sclerosis	PROK2, PKR1, PKR2	131, 160
Cardiovascular system	Cardiomyocytes survival	Impaired angiogenesis ≤3 wk	Heart failure, abdominal aortic aneurysm	PPKR1, PROK2	38, 82, 230

tions," which are associated with very poor prognosis (5, 30, 89, 154). Phan et al. (181) have recently shown that activation of the *ras* pathway plays a key role in G-CSF upregulation through activation of the Ets2 transcription factor. It has been also reported that G-CSF release from tumor cells or from the stroma, depending on the tumor type, was correlated with refractoriness to anti-VEGF antibodies in mouse models (206). Treatment with anti-G-CSF antibodies led to a substantial reduction in both circulating and tumor-associated myeloid cells in such models (206).

Consistent with a role of G-CSF signaling in regulating Bv8/PROK2 expression, Lu et al. (142) reported that the adhesion molecule CEACAM1, which is a negative regulator of G-CSFR signaling in myeloid cells (176), profoundly affects *Bv8/prok2* expression in CD11b+Gr1+ cells. Bv8/PROK-2 levels were strongly upregulated in CD11b+Gr1+ cells from CEACAM null mice implanted with B16 melanoma, and treatment with anti-G-CSF or anti-Bv8/PROK2 antibodies reduced tumor growth and angiogenesis to the levels observed in wild-type mice. The authors (142) concluded that CECAM1 is a negative regulator of myeloid cell-dependent angiogenesis through inhibition of the G-CSF-Bv8 pathway.

Kowanetz et al. (118) uncovered another critical function of the G-CSF-Bv8/PROK2 axis, facilitating tumor metastasis. They reported that the ability of 4T1 and other breast cancer cell lines to metastatize to the lungs was highly correlated with G-CSF release by the tumor cells and the resulting mobilization of Ly6G+ granulocytes, which strongly expressed Bv8/PROK2 (118). Anti-G-CSF or anti-Bv8/PROK2 antibodies significantly reduced lung metastasis. Subsequent studies independently confirmed the key role of G-CSF-mediated mobilization of granulocytes in breast cancer metastasis (28, 44).

Xin et al. (240) described another role of Bv8/PROK2 in malignant cells. Bv8/PROK2, through activation of STAT3 and downstream JAK2, had a pro-survival effect on normal and malignant human myeloid cells. Knocking down *Bv8/PROK2* in human leukemic cells reduced the activity of STAT3 and downstream genes, resulting in inhibition of in vitro growth and reduced tumorigenesis and angiogenesis in vivo (240).

Also, Curtis et al. (51) tested a small molecule Bv8/PROK2 receptor antagonist in tumor models. Administration of this inhibitor reduced growth of glioblastoma and pancreatic xenografts by suppressing angiogenesis and myeloid cell infiltration (51).

Hasnis et al. (95) have described another situation in which Bv8/PROK2 plays an important role. Tumor resistance to weekly administration of gemcitabine, a cytotoxic agent widely used to treat pancreatic cancer, was found to be related to rebound mobilization of granulocytes, and Bv8/PROK2 was highly expressed in granulocytes colonizing pancreatic tumors. Administration of anti-Bv8/PROK2 antibodies resulted in suppression of granulocyte rebound and reduced tumor regrowth, angiogenesis, and metastasis in mice treated with weekly gemcitabine (95).

Treatment with anti-Bv8/PROK2 antibodies was associated with decreased mobilization of CD11b+Gr1+ cells and a reduction in the numbers of angiogenenic islets in RIP-Tag mice (204), a transgenic model of pancreatic neuroendocrine tumorigenesis (93), indicating that anti-angiogenic ef-

Table 3. *Correlation between altered expression of the prokineticin system and human disease*

Diseases	Mutated/Altered Expression	Reference Nos.
Kallmann syndrome, idiopathic hypogonadotropic hypogonadism	PKR2, PROK2	196
Hypopituitarism	PKR2	6, 190
Hirshprung disease	PROK1, PROK2, PKR1, PKR2	172
Neuroblastoma progression	PROK1, PROK2, PKR1, PKR2	125, 174
Heart failure	PKR1, PROK2	82, 230
Abdominal aortic aneurysm	PROK2	38
Leydig cell tumors	PROK1	194
Prostate carcinogenesis	PROK1, PROK2, PKR1, PKR2	179
Polycystic ovary syndrome, ovarian hyperstimulation syndrome	PROK1	69, 144
Idiopathic recurrent pregnancy loss	PROK1, PKR!	220
Ectopic endometrium in endometriosis		227
Steroidogenic cell-derived human tumors adrenal cortex, testis, ovary	PROK1	127
Human tumors and inflammatory disorders	PROK2	251
Arthritis	PROK2	121
Tumor angiogenesis and metastasis	PROK2	207
Multiple sclerosis	PROK2	1
Parkinson's disease	PROK2	87

fects of the anti-Bv8/PROK2 antibodies are not limited to transplantable tumor models. Importantly, neutrophils infiltrating human tumors strongly express Bv8, raising the possibility that this protein may have therapeutic/diagnostic significance for some human malignancies (250). **FIGURE 7** illustrates the immunohistochemical localization of the Bv8/PROK2 protein in clusters of tumor-infiltrating neutrophils in an invasive colorectal adenocarcinoma.

As noted elsewhere in this article, Bv8/PROK2 is upregulated in synovial cells in animal models of rheumatoid arthritis (121). Angiogenesis is a key aspect of rheumatoid arthritis (reviewed in Ref. 222), and key angiogenic factors such as VEGF-A are highly expressed in human (117) and experimentally induced (157) rheumatoid arthritis. CD11b+ Gr1+ myeloid cells were found to express Bv8/PROK2 in a model of collagen-induced arthritis (121), consistent with the above-mentioned proangiogenic and proinflammatory roles of this cell type.

VII. CONCLUSIONS AND THERAPEUTIC PERSPECTIVES

As discussed throughout this article, research conducted over the last two decades has elucidated a number of important functions of this family of factors, ranging from circadian rhytms, regulation of reproductive processes, neurogenesis, angiogenesis, and inflammation. **TABLES 2 AND 3** illustrate, respectively, the biological effects of prokineticin and potential ssociated disorders and a correlation between altered expression of this system and human disease. The association of *PKR2* and *Bv8/PROK* mutations with KS provides a compelling verification of the hypothesis that this signaling system plays important roles in neurogenesis. Also, this system is implicated in the regulation of pain sensitivity associated with a number of disorders including cancer. Therefore, inhibitors of this pathway may have applications for the treatment of various types of pain.

VEGF inhibitors have been approved for the treatment of multiple malignancies and intraocular neovascular disorders, and there is an interest in improving patient outcomes and treating poor-responders (66, 67). Much recent research has focused on the microenvironment as a source of alternative proangiogenic pathways (40, 65). In particular, inflammation and infiltration by myeloid and other cell types of the innate and adaptive immune system have been strongly implicated in these processes (45). As discussed in this article, extensive preclinical data support the notion that Bv8/PROK2, produced by neutrophils and potentially other myeloid cell types, is one of the mediators of VEGF-independent angiogenesis in tumors. Indeed, the expression of Bv8/PROK2 in tumor-infiltrating neutrophils is prominent in several human tumors, including colorectal **(FIGURE 7)** and non-small-cell lung cancer (250). Therefore, clinical trials combining VEGF inhibitors with Bv8/PROK2 antagonists may have a rational foundation.

Intriguingly, recent data suggest that EG-VEGF/PROK1 might be one of the factors involved in the abnormal vascular permeability of diabetic macular edema, raising the possibility that this protein may have diagnostic/therapeutic implications in this context (27).

As discussed, there are several tantalizing clues that EG-VEGF/PROK1 also plays important functions in endocrine gland angiogenesis and in the pathophysiology of pregnancy. However, the lack of suitable rodent models has so far precluded the generation of definitive evidence. Studies in primates and possibly in humans may be required to conclusively resolve these issues.

Finally, the development of reagents that selectively target not only each ligand but also each PKR (or both) may further expand the therapeutic possibilities and potentially enable a more refined targeting of various disorders in which the prokineticin system is implicated, should the significance of the two receptors in different pathophysiological contexts become more clear.

ACKNOWLEDGMENTS

Address for reprint requests and other correspondence: L. Negri, Sapienza Univ. of Rome, Rome, Italy (e-mail: lucia.negri@uniroma1.it) or N. Ferrara, Univ. of California, San Diego, La Jolla, CA 92093 (e-mail: nferrara@ucsd.edu).

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

REFERENCES

1. Abou-Hamdan M, Costanza M, Fontana E, Di Dario M, Musio S, Congiu C, Onnis V, Lattanzi R, Radaelli M, Martinelli V, Salvadori S, Negri L, Poliani PL, Farina C, Balboni G, Steinman L, Pedotti R. Critical role for prokineticin 2 in CNS autoimmunity. *Neurol Neuroimmunol Neuroinflamm* 2: e95, 2015. doi:10.1212/NXI.0000000000000095.

2. Abreu AP, Noel SD, Xu S, Carroll RS, Latronico AC, Kaiser UB. Evidence of the importance of the first intracellular loop of prokineticin receptor 2 in receptor function. *Mol Endocrinol* 26: 1417–1427, 2012. doi:10.1210/me.2012-1102.

3. Alfaidy N. Prokineticin1 and pregnancy. *Ann Endocrinol (Paris)* 77: 101–104, 2016. doi:10.1016/j.ando.2016.04.014.

4. Alfaidy N, Hoffmann P, Boufettal H, Samouh N, Aboussaouira T, Benharouga M, Feige JJ, Brouillet S. The multiple roles of EG-VEGF/PROK1 in normal and pathological placental angiogenesis. *BioMed Res Int* 2014: 451906, 2014. doi:10.1155/2014/451906.

5. Aliper AM, Frieden-Korovkina VP, Buzdin A, Roumiantsev SA, Zhavoronkov A. A role for G-CSF and GM-CSF in nonmyeloid cancers. *Cancer Med* 3: 737–746, 2014. doi:10.1002/cam4.239.

6. Asakura Y, Muroya K, Hanakawa J, Sato T, Aida N, Narumi S, Hasegawa T, Adachi M. Combined pituitary hormone deficiency with unique pituitary dysplasia and morning glory syndrome related to a heterozygous PROKR2 mutation. *Clin Pediatr Endocrinol* 24: 27–32, 2015. doi:10.1297/cpe.24.27.

7. Balasubramanian R, Cohen DA, Klerman EB, Pignatelli D, Hall JE, Dwyer AA, Czeisler CA, Pitteloud N, Crowley WF. Absence of central circadian pacemaker abnormalities

in humans with loss of function mutation in prokineticin 2. *J Clin Endocrinol Metab* 99: E561–E566, 2014. doi:10.1210/jc.2013-2096.

8. Balasubramanian R, Plummer L, Sidis Y, Pitteloud N, Martin C, Zhou QY, Crowley WF Jr. The puzzles of the prokineticin 2 pathway in human reproduction. *Mol Cell Endocrinol* 346: 44–50, 2011. doi:10.1016/j.mce.2011.05.040.

9. Balboni G, Lazzari I, Trapella C, Negri L, Lattanzi R, Giannini E, Nicotra A, Melchiorri P, Visentin S, Nuccio CD, Salvadori S. Triazine compounds as antagonists at Bv8-prokineticin receptors. *J Med Chem* 51: 7635–7639, 2008. doi:10.1021/jm800854e.

10. Balkwill F, Mantovani A. Inflammation and cancer: back to Virchow? *Lancet* 357: 539–545, 2001. doi:10.1016/S0140-6736(00)04046-0.

11. Bălu S, Pirtea L, Gaje P, Cîmpean AM, Raica M. The immunohistochemical expression of endocrine gland-derived-VEGF (EG-VEGF) as a prognostic marker in ovarian cancer. *Rom J Morphol Embryol* 53: 479–483, 2012.

12. Battersby S, Critchley HO, Morgan K, Millar RP, Jabbour HN. Expression and regulation of the prokineticins (endocrine gland-derived vascular endothelial growth factor and Bv8) and their receptors in the human endometrium across the menstrual cycle. *J Clin Endocrinol Metab* 89: 2463–2469, 2004. doi:10.1210/jc.2003-032012.

13. Beale K, Gardiner JV, Bewick GA, Hostomska K, Patel NA, Hussain SS, Jayasena CN, Ebling FJ, Jethwa PH, Prosser HM, Lattanzi R, Negri L, Ghatei MA, Bloom SR, Dhillo WS. Peripheral administration of prokineticin 2 potently reduces food intake and body weight in mice via the brainstem. *Br J Pharmacol* 168: 403–410, 2013. doi:10.1111/j.1476-5381.2012.02191.x.

14. Bendall LJ, Bradstock KF. G-CSF: from granulopoietic stimulant to bone marrow stem cell mobilizing agent. *Cytokine Growth Factor Rev* 25: 355–367, 2014. doi:10.1016/j.cytogfr.2014.07.011.

15. Bendelac A, Fearon DT. Innate pathways that control acquired immunity. *Curr Opin Immunol* 9: 1–3, 1997. doi:10.1016/S0952-7915(97)80151-3.

16. Boehm U, Bouloux PM, Dattani MT, de Roux N, Dodé C, Dunkel L, Dwyer AA, Giacobini P, Hardelin JP, Juul A, Maghnie M, Pitteloud N, Prevot V, Raivio T, Tena-Sempere M, Quinton R, Young J. European Consensus Statement on congenital hypogonadotropic hypogonadism–pathogenesis, diagnosis and treatment. *Nat Rev Endocrinol* 11: 547–564, 2015. doi:10.1038/nrendo.2015.112.

17. Boisbouvier J, Albrand JP, Blackledge M, Jaquinod M, Schweitz H, Lazdunski M, Marion D. A structural homologue of colipase in black mamba venom revealed by NMR floating disulphide bridge analysis. *J Mol Biol* 283: 205–219, 1998. doi:10.1006/jmbi.1998.2057.

18. Boyce S, Wyatt A, Webb JK, O'Donnell R, Mason G, Rigby M, Sirinathsinghji D, Hill RG, Rupniak NM. Selective NMDA NR2B antagonists induce antinociception without motor dysfunction: correlation with restricted localisation of NR2B subunit in dorsal horn. *Neuropharmacology* 38: 611–623, 1999. doi:10.1016/S0028-3908(98)00218-4.

19. Braun SM, Jessberger S. Adult neurogenesis: mechanisms and functional significance. *Development* 141: 1983–1986, 2014. doi:10.1242/dev.104596.

20. Brouillet S, Hoffmann P, Benharouga M, Salomon A, Schaal JP, Feige JJ, Alfaidy N. Molecular characterization of EG-VEGF-mediated angiogenesis: differential effects on microvascular and macrovascular endothelial cells. *Mol Biol Cell* 21: 2832–2843, 2010. doi:10.1091/mbc.E10-01-0059.

21. Brouillet S, Hoffmann P, Feige JJ, Alfaidy N. EG-VEGF: a key endocrine factor in placental development. *Trends Endocrinol Metab* 23: 501–508, 2012. doi:10.1016/j.tem.2012.05.006.

22. Brouillet S, Murthi P, Hoffmann P, Salomon A, Sergent F, De Mazancourt P, Dakouane-Giudicelli M, Dieudonné MN, Rozenberg P, Vaiman D, Barbaux S, Benharouga M, Feige JJ, Alfaidy N. EG-VEGF controls placental growth and survival in normal and pathological pregnancies: case of fetal growth restriction (FGR). *Cell Mol Life Sci* 70: 511–525, 2013. doi:10.1007/s00018-012-1141-z.

23. Bullock CM, Li JD, Zhou QY. Structural determinants required for the bioactivities of prokineticins and identification of prokineticin receptor antagonists. *Mol Pharmacol* 65: 582–588, 2004. doi:10.1124/mol.65.3.582.

24. Burton KJ, Li X, Li B, Cheng MY, Urbanski HF, Zhou QY. Expression of prokineticin 2 and its receptor in the macaque monkey brain. *Chronobiol Int* 33: 191–199, 2016. doi:10.3109/07420528.2015.1125361.

25. Burton KJ, Li X, Li JD, Hu WP, Zhou QY. Rhythmic Trafficking of TRPV2 in the Suprachiasmatic Nucleus Is Regulated by Prokineticin 2 Signaling. *J Circadian Rhythms* 13: 2, 2015. doi:10.5334/jcr.ad.

26. Caioli S, Severini C, Ciotti T, Florenzano F, Pimpinella D, Petrocchi Passeri P, Balboni G, Polisca P, Lattanzi R, Nisticò R, Negri L, Zona C. Prokineticin system modulation as a new target to counteract the amyloid beta toxicity induced by glutamatergic alterations in an in vitro model of Alzheimer's disease. *Neuropharmacology* 116: 82–97, 2017. doi:10.1016/j.neuropharm.2016.12.012.

27. Campochiaro PA, Hafiz G, Mir TA, Scott AW, Zimmer-Galler I, Shah SM, Wenick AS, Brady CJ, Han I, He L, Channa R, Poon D, Meyerle C, Aronow MB, Sodhi A, Handa JT, Kherani S, Han Y, Sophie R, Wang G, Qian J. Pro-permeability Factors in Diabetic Macular Edema; the Diabetic Macular Edema Treated With Ozurdex Trial. *Am J Ophthalmol* 168: 13–23, 2016. doi:10.1016/j.ajo.2016.04.017.

28. Casbon AJ, Reynaud D, Park C, Khuc E, Gan DD, Schepers K, Passegué E, Werb Z. Invasive breast cancer reprograms early myeloid differentiation in the bone marrow to generate immunosuppressive neutrophils. *Proc Natl Acad Sci USA* 112: E566–E575, 2015. doi:10.1073/pnas.1424927112.

29. Castelli M, Amodeo G, Negri L, Lattanzi R, Maftei D, Gotti C, Pistillo F, Onnis V, Congu C, Panerai AE, Sacerdote P, Franchi S. Antagonism of the Prokineticin System Prevents and Reverses Allodynia and Inflammation in a Mouse Model of Diabetes. *PLoS One* 11: e0146259, 2016. doi:10.1371/journal.pone.0146259.

30. Chakraborty S, Keenportz B, Woodward S, Anderson J, Colan D. Paraneoplastic leukemoid reaction in solid tumors. *Am J Clin Oncol* 38: 326–330, 2015. doi:10.1097/COC.0b013e3182a530dd.

31. Chaly AL, Srisai D, Gardner EE, Sebag JA. The Melanocortin Receptor Accessory Protein 2 promotes food intake through inhibition of the Prokineticin Receptor-1. *eLife* 5: e12397, 2016. doi:10.7554/eLife.12397.

32. Chen J, Kuei C, Sutton S, Wilson S, Yu J, Kamme F, Mazur C, Lovenberg T, Liu C. Identification and pharmacological characterization of prokineticin 2 beta as a selective ligand for prokineticin receptor 1. *Mol Pharmacol* 67: 2070–2076, 2005. doi:10.1124/mol.105.011619.

33. Chen T, Xue Y, Zhou M, Shaw C. Molecular cloning of mRNA from toad granular gland secretion and lyophilized skin: identification of Bo8–a novel prokineticin from *Bombina orientalis*. *Peptides* 26: 377–383, 2005. doi:10.1016/j.peptides.2004.10.021.

34. Cheng MY, Bittman EL, Hattar S, Zhou QY. Regulation of prokineticin 2 expression by light and the circadian clock. *BMC Neurosci* 6: 17, 2005. doi:10.1186/1471-2202-6-17.

35. Cheng MY, Bullock CM, Li C, Lee AG, Bermak JC, Belluzzi J, Weaver DR, Leslie FM, Zhou QY. Prokineticin 2 transmits the behavioural circadian rhythm of the suprachiasmatic nucleus. *Nature* 417: 405–410, 2002. doi:10.1038/417405a.

36. Cheng MY, Lee AG, Culbertson C, Sun G, Talati RK, Manley NC, Li X, Zhao H, Lyons DM, Zhou QY, Steinberg GK, Sapolsky RM. Prokineticin 2 is an endangering mediator of cerebral ischemic injury. *Proc Natl Acad Sci USA* 109: 5475–5480, 2012. doi:10.1073/pnas.1113363109.

37. Cheng MY, Leslie FM, Zhou QY. Expression of prokineticins and their receptors in the adult mouse brain. *J Comp Neurol* 498: 796–809, 2006. doi:10.1002/cne.21087.

38. Choke E, Cockerill GW, Laing K, Dawson J, Wilson WR, Loftus IM, Thompson MM. Whole genome-expression profiling reveals a role for immune and inflammatory response in abdominal aortic aneurysm rupture. *Eur J Vasc Endovasc Surg* 37: 305–310, 2009. doi:10.1016/j.ejvs.2008.11.017.

39. Chung AS, Ferrara N. Developmental and pathological angiogenesis. *Annu Rev Cell Dev Biol* 27: 563–584, 2011. doi:10.1146/annurev-cellbio-092910-154002.

40. Chung AS, Lee J, Ferrara N. Targeting the tumour vasculature: insights from physiological angiogenesis. *Nat Rev Cancer* 10: 505–514, 2010. doi:10.1038/nrc2868.

41. Chung AS, Wu X, Zhuang G, Ngu H, Kasman I, Zhang J, Vernes JM, Jiang Z, Meng YG, Peale FV, Ouyang W, Ferrara N. An interleukin-17-mediated paracrine network promotes tumor resistance to anti-angiogenic therapy. *Nat Med* 19: 1114–1123, 2013. doi:10.1038/nm.3291.

42. Clark HF, Gurney AL, Abaya E, Baker K, Baldwin D, Brush J, Chen J, Chow B, Chui C, Crowley C, Currell B, Deuel B, Dowd P, Eaton D, Foster J, Grimaldi C, Gu Q, Hass PE, Heldens S, Huang A, Kim HS, Klimowski L, Jin Y, Johnson S, Lee J, Lewis L, Liao D, Mark M, Robbie E, Sanchez C, Schoenfeld J, Seshagiri S, Simmons L, Singh J, Smith V, Stinson J, Vagts A, Vandlen R, Watanabe C, Wieand D, Woods K, Xie MH, Yansura D,

Yi S, Yu G, Yuan J, Zhang M, Zhang Z, Goddard A, Wood WI, Godowski P, Gray A. The secreted protein discovery initiative (SPDI), a large-scale effort to identify novel human secreted and transmembrane proteins: a bioinformatics assessment. *Genome Res* 13: 2265–2270, 2003. doi:10.1101/gr.1293003.

43. Clement PB. Histology of the ovary. *Am J Surg Pathol* 11: 277–303, 1987. doi:10.1097/00000478-198704000-00006.

44. Coffelt SB, Kersten K, Doornebal CW, Weiden J, Vrijland K, Hau CS, Verstegen NJM, Ciampricotti M, Hawinkels LJAC, Jonkers J, de Visser KE. IL-17-producing $\gamma\delta$ T cells and neutrophils conspire to promote breast cancer metastasis. *Nature* 522: 345–348, 2015. doi:10.1038/nature14282.

45. Coffelt SB, Lewis CE, Naldini L, Brown JM, Ferrara N, De Palma M. Elusive identities and overlapping phenotypes of proangiogenic myeloid cells in tumors. *Am J Pathol* 176: 1564–1576, 2010. doi:10.2353/ajpath.2010.090786.

46. Constantinescu CS, Farooqi N, O'Brien K, Gran B. Experimental autoimmune encephalomyelitis (EAE) as a model for multiple sclerosis (MS). *Br J Pharmacol* 164: 1079–1106, 2011. doi:10.1111/j.1476-5381.2011.01302.x.

47. Cook IH, Evans J, Maldonado-Pérez D, Critchley HO, Sales KJ, Jabbour HN. Prokineticin-1 (PROK1) modulates interleukin (IL)-11 expression via prokineticin receptor 1 (PROKR1) and the calcineurin/NFAT signalling pathway. *Mol Hum Reprod* 16: 158–169, 2010. doi:10.1093/molehr/gap084.

48. Costigan M, Scholz J, Woolf CJ. Neuropathic pain: a maladaptive response of the nervous system to damage. *Annu Rev Neurosci* 32: 1–32, 2009. doi:10.1146/annurev.neuro.051508.135531.

49. Cottrell GT, Zhou QY, Ferguson AV. Prokineticin 2 modulates the excitability of subfornical organ neurons. *J Neurosci* 24: 2375–2379, 2004. doi:10.1523/JNEUROSCI.5187-03.2004.

50. Coussens LM, Werb Z. Inflammation and cancer. *Nature* 420: 860–867, 2002. doi:10.1038/nature01322.

51. Curtis VF, Wang H, Yang P, McLendon RE, Li X, Zhou QY, Wang XF. A PK2/Bv8/PROK2 antagonist suppresses tumorigenic processes by inhibiting angiogenesis in glioma and blocking myeloid cell infiltration in pancreatic cancer. *PLoS One* 8: e54916, 2013. doi:10.1371/journal.pone.0054916.

52. De Felice M, Melchiorri P, Ossipov MH, Vanderah TW, Porreca F, Negri L. Mechanisms of Bv8-induced biphasic hyperalgesia: increased excitatory transmitter release and expression. *Neurosci Lett* 521: 40–45, 2012. doi:10.1016/j.neulet.2012.05.055.

53. De Novellis V, Negri L, Lattanzi R, Rossi F, Palazzo E, Marabese I, Giannini E, Vita D, Melchiorri P, Maione S. The prokineticin receptor agonist Bv8 increases GABA release in the periaqueductal grey and modifies RVM cell activities and thermoceptive reflexes in the rat. *Eur J Neurosci* 26: 3068–3078, 2007. doi:10.1111/j.1460-9568.2007.05910.x.

54. Diaz-Montero CM, Salem ML, Nishimura MI, Garrett-Mayer E, Cole DJ, Montero AJ. Increased circulating myeloid-derived suppressor cells correlate with clinical cancer stage, metastatic tumor burden, and doxorubicin-cyclophosphamide chemotherapy. *Cancer Immunol Immunother* 58: 49–59, 2009. doi:10.1007/s00262-008-0523-4.

55. Dodé C, Rondard P. PROK2/PROKR2 Signaling and Kallmann Syndrome. *Front Endocrinol (Lausanne)* 4: 19, 2013.

56. Dodé C, Teixeira L, Levilliers J, Fouveaut C, Bouchard P, Kottler ML, Lespinasse J, Lienhardt-Roussie A, Mathieu M, Moerman A, Morgan G, Murat A, Toublanc JE, Wolczynski S, Delpech M, Petit C, Young J, Hardelin JP. Kallmann syndrome: mutations in the genes encoding prokineticin-2 and prokineticin receptor-2. *PLoS Genet* 2: e175, 2006. doi:10.1371/journal.pgen.0020175.

57. Dormishian M, Turkeri G, Urayama K, Nguyen TL, Boulberdaa M, Messaddeq N, Renault G, Henrion D, Nebigil CG. Prokineticin receptor-1 is a new regulator of endothelial insulin uptake and capillary formation to control insulin sensitivity and cardiovascular and kidney functions. *J Am Heart Assoc* 2: e000411, 2013. doi:10.1161/JAHA.113.000411.

58. Dorsch M, Qiu Y, Soler D, Frank N, Duong T, Goodearl A, O'Neil S, Lora J, Fraser CC. PK1/EG-VEGF induces monocyte differentiation and activation. *J Leukoc Biol* 78: 426–434, 2005. doi:10.1189/jlb.0205061.

59. Dourron NE, Williams DB. Prevention and treatment of ovarian hyperstimulation syndrome. *Semin Reprod Endocrinol* 14: 355–365, 1996. doi:10.1055/s-2008-1067980.

60. Dragneva G, Korpisalo P, Ylä-Herttuala S. Promoting blood vessel growth in ischemic diseases: challenges in translating preclinical potential into clinical success. *Dis Model Mech* 6: 312–322, 2013. doi:10.1242/dmm.010413.

61. Dunand C, Hoffmann P, Sapin V, Blanchon L, Salomon A, Sergent F, Benharouga M, Sabra S, Guibourdenche J, Lye SJ, Feige JJ, Alfaidy N. Endocrine gland-derived endothelial growth factor (EG-VEGF) is a potential novel regulator of human parturition. *Biol Reprod* 91: 73, 2014. doi:10.1095/biolreprod.114.119990.

62. Eddie SL, Childs AJ, Kinnell HL, Brown P, Jabbour HN, Anderson RA. Prokineticin Ligands and Receptors Are Expressed in the Human Fetal Ovary and Regulate Germ Cell Expression of COX2. *J Clin Endocrinol Metab* 100: E1197–E1205, 2015. doi:10.1210/jc.2015-2330.

63. Evans J, Catalano RD, Morgan K, Critchley HO, Millar RP, Jabbour HN. Prokineticin 1 signaling and gene regulation in early human pregnancy. *Endocrinology* 149: 2877–2887, 2008. doi:10.1210/en.2007-1633.

64. Falk S, Dickenson AH. Pain and nociception: mechanisms of cancer-induced bone pain. *J Clin Oncol* 32: 1647–1654, 2014. doi:10.1200/JCO.2013.51.7219.

65. Ferrara N. Pathways mediating VEGF-independent tumor angiogenesis. *Cytokine Growth Factor Rev* 21: 21–26, 2010. doi:10.1016/j.cytogfr.2009.11.003.

66. Ferrara N. Vascular endothelial growth factor: basic science and clinical progress. *Endocr Rev* 25: 581–611, 2004. doi:10.1210/er.2003-0027.

67. Ferrara N, Adamis AP. Ten years of anti-vascular endothelial growth factor therapy. *Nat Rev Drug Discov* 15: 385–403, 2016. doi:10.1038/nrd.2015.17.

68. Ferrara N, Carver-Moore K, Chen H, Dowd M, Lu L, O'Shea KS, Powell-Braxton L, Hillan KJ, Moore MW. Heterozygous embryonic lethality induced by targeted inactivation of the VEGF gene. *Nature* 380: 439–442, 1996. doi:10.1038/380439a0.

69. Ferrara N, Frantz G, LeCouter J, Dillard-Telm L, Pham T, Draksharapu A, Giordano T, Peale F. Differential expression of the angiogenic factor genes vascular endothelial growth factor (VEGF) and endocrine gland-derived VEGF in normal and polycystic human ovaries. *Am J Pathol* 162: 1881–1893, 2003. doi:10.1016/S0002-9440(10)64322-2.

70. Ferrara N, Gerber HP, LeCouter J. The biology of VEGF and its receptors. *Nat Med* 9: 669–676, 2003. doi:10.1038/nm0603-669.

71. Ferrara N, LeCouter J, Lin R, Peale F. EG-VEGF and Bv8: a novel family of tissue-restricted angiogenic factors. *Biochim Biophys Acta* 1654: 69–78, 2004.

72. Franchi S, Giannini E, Lattuada D, Lattanzi R, Tian H, Melchiorri P, Negri L, Panerai AE, Sacerdote P. The prokineticin receptor agonist Bv8 decreases IL-10 and IL-4 production in mice splenocytes by activating prokineticin receptor-1. *BMC Immunol* 9: 60, 2008. doi:10.1186/1471-2172-9-60.

73. Fraser HM, Bell J, Wilson H, Taylor PD, Morgan K, Anderson RA, Duncan WC. Localization and quantification of cyclic changes in the expression of endocrine gland vascular endothelial growth factor in the human corpus luteum. *J Clin Endocrinol Metab* 90: 427–434, 2005. doi:10.1210/jc.2004-0843.

74. Fry M, Cottrell GT, Ferguson AV. Prokineticin 2 influences subfornical organ neurons through regulation of MAP kinase and the modulation of sodium channels. *Am J Physiol Regul Integr Comp Physiol* 295: R848–R856, 2008. doi:10.1152/ajpregu.00779.2007.

75. Gabrilovich DI. Myeloid-Derived Suppressor Cells. *Cancer Immunol Res* 5: 3–8, 2017. doi:10.1158/2326-6066.CIR-16-0297.

76. Galer BS, Gianas A, Jensen MP. Painful diabetic polyneuropathy: epidemiology, pain description, and quality of life. *Diabetes Res Clin Pract* 47: 123–128, 2000. doi:10.1016/S0168-8227(99)00112-6.

77. Gao MZ, Zhao XM, Lin Y, Sun ZG, Zhang HQ. Effects of EG-VEGF, VEGF and TGF-β1 on pregnancy outcome in patients undergoing IVF-ET treatment. *J Assist Reprod Genet* 29: 1091–1096, 2012. doi:10.1007/s10815-012-9833-8.

78. Gao MZ, Zhao XM, Sun ZG, Hong Y, Zhao LW, Zhang HQ. Endocrine gland-derived vascular endothelial growth factor concentrations in follicular fluid and serum may predict ovarian hyperstimulation syndrome in women undergoing controlled ovarian hyperstimulation. *Fertil Steril* 95: 673–678, 2011. doi:10.1016/j.fertnstert.2010.09.044.

79. Gao YJ, Ji RR. Chemokines, neuronal-glial interactions, and central processing of neuropathic pain. *Pharmacol Ther* 126: 56–68, 2010. doi:10.1016/j.pharmthera.2010.01.002.

80. Gardiner JV, Bataveljic A, Patel NA, Bewick GA, Roy D, Campbell D, Greenwood HC, Murphy KG, Hameed S, Jethwa PH, Ebling FJ, Vickers SP, Cheetham S, Ghatei MA, Bloom SR, Dhillo WS. Prokineticin 2 is a hypothalamic neuropeptide that potently inhibits food intake. *Diabetes* 59: 397–406, 2010. doi:10.2337/db09-1198.

81. Garlanda C, Parravicini C, Sironi M, De Rossi M, Wainstok de Calmanovici R, Carozzi F, Bussolino F, Colotta F, Mantovani A, Vecchi A. Progressive growth in immunodeficient mice and host cell recruitment by mouse endothelial cells transformed by polyoma middle-sized T antigen: implications for the pathogenesis of opportunistic vascular tumors. *Proc Natl Acad Sci USA* 91: 7291–7295, 1994. doi:10.1073/pnas.91.15.7291.

82. Gasser A, Brogi S, Urayama K, Nishi T, Kurose H, Tafi A, Ribeiro N, Désaubry L, Nebigil CG. Discovery and cardioprotective effects of the first non-Peptide agonists of the G protein-coupled prokineticin receptor-1. *PLoS One* 10: e0121027, 2015. doi:10.1371/journal.pone.0121027.

83. Gerber HP, Malik AK, Solar GP, Sherman D, Liang XH, Meng G, Hong K, Marsters JC, Ferrara N. VEGF regulates haematopoietic stem cell survival by an internal autocrine loop mechanism. *Nature* 417: 954–958, 2002. doi:10.1038/nature00821.

84. Giannini E, Lattanzi R, Nicotra A, Campese AF, Grazioli P, Screpanti I, Balboni G, Salvadori S, Sacerdote P, Negri L. The chemokine Bv8/prokineticin 2 is up-regulated in inflammatory granulocytes and modulates inflammatory pain. *Proc Natl Acad Sci USA* 106: 14646–14651, 2009. doi:10.1073/pnas.0903720106.

85. Glinka A, Wu W, Delius H, Monaghan AP, Blumenstock C, Niehrs C. Dickkopf-1 is a member of a new family of secreted proteins and functions in head induction. *Nature* 391: 357–362, 1998. doi:10.1038/34848.

86. Goi T, Nakazawa T, Hirono Y, Yamaguchi A. Prokineticin 1 expression in gastrointestinal tumors. *Anticancer Res* 33: 5311–5315, 2013.

87. Gordon R, Neal ML, Luo J, Langley MR, Harischandra DS, Panicker N, Charli A, Jin H, Anantharam V, Woodruff TM, Zhou QY, Kanthasamy AG, Kanthasamy A. Prokineticin-2 upregulation during neuronal injury mediates a compensatory protective response against dopaminergic neuronal degeneration. *Nat Commun* 7: 12932, 2016. doi:10.1038/ncomms12932.

88. Goverman J. Autoimmune T cell responses in the central nervous system. *Nat Rev Immunol* 9: 393–407, 2009. doi:10.1038/nri2550.

89. Granger JM, Kontoyiannis DP. Etiology and outcome of extreme leukocytosis in 758 nonhematologic cancer patients: a retrospective, single-institution study. *Cancer* 115: 3919–3923, 2009. doi:10.1002/cncr.24480.

90. Guida F, Luongo L, Marmo F, Romano R, Iannotta M, Napolitano F, Belardo C, Marabese I, D'Aniello A, De Gregorio D, Rossi F, Piscitelli F, Lattanzi R, de Bartolomeis A, Usiello A, Di Marzo V, de Novellis V, Maione S. Palmitoylethanolamide reduces pain-related behaviors and restores glutamatergic synapses homeostasis in the medial prefrontal cortex of neuropathic mice. *Mol Brain* 8: 47, 2015. doi:10.1186/s13041-015-0139-5.

91. Guilini C, Urayama K, Turkeri G, Dedeoglu DB, Kurose H, Messaddeq N, Nebigil CG. Divergent roles of prokineticin receptors in the endothelial cells: angiogenesis and fenestration. *Am J Physiol Heart Circ Physiol* 298: H844–H852, 2010. doi:10.1152/ajpheart.00898.2009.

92. Han BY, Li LL, Wang CZ, Guo QH, Lv ZH, Mu YM, Dou JT. Correlation between Pituitary Stalk Interruption Syndrome and Prokineticin Receptor 2 and Prokineticin 2 Mutations. *Zhongguo Yi Xue Ke Xue Yuan Xue Bao* 38: 37–41, 2016.

93. Hanahan D. Heritable formation of pancreatic beta-cell tumours in transgenic mice expressing recombinant insulin/simian virus 40 oncogenes. *Nature* 315: 115–122, 1985. doi:10.1038/315115a0.

94. Hang LH, Luo H, Li SN, Shu WW, Chen Z, Chen YF, Yuan JF, Shi LL, Shao DH. Involvement of Spinal Bv8/Prokineticin 2 in a Rat Model of Cancer-Induced Bone Pain. *Basic Clin Pharmacol Toxicol* 117: 180–185, 2015. doi:10.1111/bcpt.12386.

95. Hasnis E, Alishekevitz D, Gingis-Veltski S, Bril R, Fremder E, Voloshin T, Raviv Z, Karban A, Shaked Y. Anti-Bv8 antibody and metronomic gemcitabine improve pancreatic adenocarcinoma treatment outcome following weekly gemcitabine therapy. *Neoplasia* 16: 501–510, 2014. doi:10.1016/j.neo.2014.05.011.

96. Hastings MH. Circadian rhythms: a gut feeling for time. *Nature* 417: 391–392, 2002. doi:10.1038/417391a.

97. Heck D, Wortmann S, Kraus L, Ronchi CL, Sinnott RO, Fassnacht M, Sbiera S. Role of Endocrine Gland-Derived Vascular Endothelial Growth Factor (EG-VEGF) and Its Receptors in Adrenocortical Tumors. *Horm Cancer* 6: 225–236, 2015. doi:10.1007/s12672-015-0236-z.

98. Hodgen GD. The dominant ovarian follicle. *Fertil Steril* 38: 281–300, 1982.

99. Hoffmann P, Feige JJ, Alfaidy N. Expression and oxygen regulation of endocrine gland-derived vascular endothelial growth factor/prokineticin-1 and its receptors in human placenta during early pregnancy. *Endocrinology* 147: 1675–1684, 2006. doi:10.1210/en.2005-0912.

100. Hoffmann P, Feige JJ, Alfaidy N. Placental expression of EG-VEGF and its receptors PKR1 (prokineticin receptor-1) and PKR2 throughout mouse gestation. *Placenta* 28: 1049–1058, 2007. doi:10.1016/j.placenta.2007.03.008.

101. Hoffmann P, Saoudi Y, Benharouga M, Graham CH, Schaal JP, Mazouni C, Feige JJ, Alfaidy N. Role of EG-VEGF in human placentation: physiological and pathological implications. *J Cell Mol Med* 13, 8B: 2224–2235, 2009. doi:10.1111/j.1582-4934.2008.00554.x.

102. Hoffmann T, Negri L, Maftei D, Lattanzi R, Reeh PW. The prokineticin Bv8 sensitizes cutaneous terminals of female mice to heat. *Eur J Pain* 20: 1326–1334, 2016. doi:10.1002/ejp.857.

103. Hu WP, Li JD, Zhang C, Boehmer L, Siegel JM, Zhou QY. Altered circadian and homeostatic sleep regulation in prokineticin 2-deficient mice. *Sleep* 30: 247–256, 2007.

104. Hu WP, Zhang C, Li JD, Luo ZD, Amadesi S, Bunnett N, Zhou QY. Impaired pain sensation in mice lacking prokineticin 2. *Mol Pain* 2: 35, 2006. doi:10.1186/1744-8069-2-35.

105. Ingves MV, Ferguson AV. Prokineticin 2 modulates the excitability of area postrema neurons in vitro in the rat. *Am J Physiol Regul Integr Comp Physiol* 298: R617–R626, 2010. doi:10.1152/ajpregu.00620.2009.

106. Ito H, Noda K, Yoshida K, Otani K, Yoshiga M, Oto Y, Saito S, Kurosaka D. Prokineticin 2 antagonist, PKRA7 suppresses arthritis in mice with collagen-induced arthritis. *BMC Musculoskelet Disord* 17: 387, 2016. doi:10.1186/s12891-016-1243-0.

107. Iwasa T, Matsuzaki T, Tungalagsuvd A, Munkhzaya M, Kawami T, Yamasaki M, Murakami M, Kato T, Kuwahara A, Yasui T, Irahara M. Changes in the responsiveness of hypothalamic PK2 and PKR1 gene expression to fasting in developing male rats. *Int J Dev Neurosci* 38: 87–90, 2014. doi:10.1016/j.ijdevneu.2014.08.004.

108. Jilek A, Engel E, Beier D, Lepperdinger G. Murine Bv8 gene maps near a synteny breakpoint of mouse chromosome 6 and human 3p21. *Gene* 256: 189–195, 2000. doi:10.1016/S0378-1119(00)00355-3.

109. Joubert FJ, Strydom DJ. Snake venom. The amino acid sequence of protein A from *Dendroaspis polylepis polylepis* (black mamba) venom. *Hoppe Seylers Z Physiol Chem* 361: 1787–1794, 1980. doi:10.1515/bchm2.1980.361.2.1787.

110. Kaser A, Winklmayr M, Lepperdinger G, Kreil G. The AVIT protein family. Secreted cysteine-rich vertebrate proteins with diverse functions. *EMBO Rep* 4: 469–473, 2003. doi:10.1038/sj.embor.embor830.

111. Kemmann E, Tavakoli F, Shelden RM, Jones JR. Induction of ovulation with menotropins in women with polycystic ovary syndrome. *Am J Obstet Gynecol* 141: 58–64, 1981. doi:10.1016/0002-9378(81)90675-X.

112. Keramidas M, Faudot C, Cibiel A, Feige JJ, Thomas M. Mitogenic functions of endocrine gland-derived vascular endothelial growth factor and *Bombina variegata* 8 on steroidogenic adrenocortical cells. *J Endocrinol* 196: 473–482, 2008. doi:10.1677/JOE-07-0255.

113. Khusal KG, Tonelli RR, Mattos EC, Soares CO, Di Genova BM, Juliano MA, Urias U, Colli W, Alves MJ. Prokineticin receptor identified by phage display is an entry receptor for *Trypanosoma cruzi* into mammalian cells. *Parasitol Res* 114: 155–165, 2015. doi:10.1007/s00436-014-4172-6.

114. Kishi T, Kitajima T, Tsunoka T, Okumura T, Ikeda M, Okochi T, Kinoshita Y, Kawashima K, Yamanouchi Y, Ozaki N, Iwata N. Possible association of prokineticin 2 receptor gene (PROKR2) with mood disorders in the Japanese population. *Neuromolecular Med* 11: 114–122, 2009. doi:10.1007/s12017-009-8067-0.

115. Kisliouk T, Friedman A, Klipper E, Zhou QY, Schams D, Alfaidy N, Meidan R. Expression pattern of prokineticin 1 and its receptors in bovine ovaries during the estrous

cycle: involvement in corpus luteum regression and follicular atresia. *Biol Reprod* 76: 749–758, 2007. doi:10.1095/biolreprod.106.054734.

116. Kisliouk T, Podlovni H, Meidan R. Unique expression and regulatory mechanisms of EG-VEGF/prokineticin-1 and its receptors in the corpus luteum. *Ann Anat* 187: 529–537, 2005. doi:10.1016/j.aanat.2005.07.005.

117. Koch AE, Harlow LA, Haines GK, Amento EP, Unemori EN, Wong W-L, Pope RM, Ferrara N. Vascular endothelial growth factor. A cytokine modulating endothelial function in rheumatoid arthritis. *J Immunol* 152: 4149–4156, 1994.

118. Kowanetz M, Wu X, Lee J, Tan M, Hagenbeek T, Qu X, Yu L, Ross J, Korsisaari N, Cao T, Bou-Reslan H, Kallop D, Weimer R, Ludlam MJ, Kaminker JS, Modrusan Z, van Bruggen N, Peale FV, Carano R, Meng YG, Ferrara N. Granulocyte-colony stimulating factor promotes lung metastasis through mobilization of Ly6G+Ly6C+ granulocytes. *Proc Natl Acad Sci USA* 107: 21248–21255, 2010. doi:10.1073/pnas.1015855107.

119. Koyama Y, Kiyo-oka M, Osakada M, Horiguchi N, Shintani N, Ago Y, Kakuda M, Baba A, Matsuda T. Expression of prokineticin receptors in mouse cultured astrocytes and involvement in cell proliferation. *Brain Res* 1112: 65–69, 2006. doi:10.1016/j.brainres.2006.07.013.

120. Kubo A, Sujino M, Masumoto KH, Fujioka A, Terashima T, Shigeyoshi Y, Nagano M. Profiles of Periglomerular Cells in the Olfactory Bulb of Prokineticin Type 2 Receptor-deficient Mice. *Acta Histochem Cytochem* 50: 95–104, 2017. doi:10.1267/ahc.17001.

121. Kurosaka D, Noda K, Yoshida K, Furuya K, Ukichi T, Takahashi E, Yanagimachi M, Kingetsu I, Saito S, Yamada A. Elevation of *Bombina variegata* peptide 8 in mice with collagen-induced arthritis. *BMC Musculoskelet Disord* 10: 45, 2009. doi:10.1186/1471-2474-10-45.

122. Lambert CM, Machida KK, Smale L, Nunez AA, Weaver DR. Analysis of the prokineticin 2 system in a diurnal rodent, the unstriped Nile grass rat (*Arvicanthis niloticus*). *J Biol Rhythms* 20: 206–218, 2005. doi:10.1177/0748730405275135.

123. Landucci E, Lattanzi R, Gerace E, Scartabelli T, Balboni G, Negri L, Pellegrini-Giampietro DE. Prokineticins are neuroprotective in models of cerebral ischemia and ischemic tolerance in vitro. *Neuropharmacology* 108: 39–48, 2016. doi:10.1016/j.neuropharm.2016.04.043.

124. Lattanzi R, Sacerdote P, Franchi S, Canestrelli M, Miele R, Barra D, Visentin S, DeNuccio C, Porreca F, De Felice M, Guida F, Luongo L, de Novellis V, Maione S, Negri L. Pharmacological activity of a Bv8 analogue modified in position 24. *Br J Pharmacol* 166: 950–963, 2012. doi:10.1111/j.1476-5381.2011.01797.x.

125. Lau ST, Hansford LM, Chan WK, Chan GC, Wan TS, Wong KK, Kaplan DR, Tam PK, Ngan ES. Prokineticin signaling is required for the maintenance of a de novo population of c-KIT$^+$ cells to sustain neuroblastoma progression. *Oncogene* 34: 1019–1034, 2015. doi:10.1038/onc.2014.24.

126. LeCouter J, Ferrara N. EG-VEGF and Bv8. a novel family of tissue-selective mediators of angiogenesis, endothelial phenotype, and function. *Trends Cardiovasc Med* 13: 276–282, 2003. doi:10.1016/S1050-1738(03)00110-5.

127. LeCouter J, Kowalski J, Foster J, Hass P, Zhang Z, Dillard-Telm L, Frantz G, Rangell L, DeGuzman L, Keller G-A, Peale F, Gurney A, Hillan KJ, Ferrara N. Identification of an angiogenic mitogen selective for endocrine gland endothelium. *Nature* 412: 877–884, 2001. doi:10.1038/35091000.

128. LeCouter J, Lin R, Ferrara N. Endocrine gland-derived VEGF and the emerging hypothesis of organ-specific regulation of angiogenesis. *Nat Med* 8: 913–917, 2002. doi:10.1038/nm0902-913.

129. LeCouter J, Lin R, Frantz G, Zhang Z, Hillan K, Ferrara N. Mouse endocrine gland-derived vascular endothelial growth factor: a distinct expression pattern from its human ortholog suggests different roles as a regulator of organ-specific angiogenesis. *Endocrinology* 144: 2606–2616, 2003. doi:10.1210/en.2002-0146.

130. LeCouter J, Lin R, Tejada M, Frantz G, Peale F, Hillan KJ, Ferrara N. The endocrine-gland-derived VEGF homologue Bv8 promotes angiogenesis in the testis: localization of Bv8 receptors to endothelial cells. *Proc Natl Acad Sci USA* 100: 2685–2690, 2003. doi:10.1073/pnas.0337667100.

131. LeCouter J, Zlot C, Tejada M, Peale F, Ferrara N. Bv8 and endocrine gland-derived vascular endothelial growth factor stimulate hematopoiesis and hematopoietic cell mobilization. *Proc Natl Acad Sci USA* 101: 16813–16818, 2004. doi:10.1073/pnas.0407697101.

132. Levit A, Yarnitzky T, Wiener A, Meidan R, Niv MY. Modeling of human prokineticin receptors: interactions with novel small-molecule binders and potential off-target drugs. *PLoS One* 6: e27990, 2011. doi:10.1371/journal.pone.0027990.

133. Li JD, Hu WP, Boehmer L, Cheng MY, Lee AG, Jilek A, Siegel JM, Zhou QY. Attenuated circadian rhythms in mice lacking the prokineticin 2 gene. *J Neurosci* 26: 11615–11623, 2006. doi:10.1523/JNEUROSCI.3679-06.2006.

134. Li JD, Hu WP, Zhou QY. The circadian output signals from the suprachiasmatic nuclei. *Prog Brain Res* 199: 119–127, 2012. doi:10.1016/B978-0-444-59427-3.00028-9.

135. Li JD, Hu WP, Zhou QY. Disruption of the circadian output molecule prokineticin 2 results in anxiolytic and antidepressant-like effects in mice. *Neuropsychopharmacology* 34: 367–373, 2009. doi:10.1038/npp.2008.61.

136. Li M, Bullock CM, Knauer DJ, Ehlert FJ, Zhou QY. Identification of two prokineticin cDNAs: recombinant proteins potently contract gastrointestinal smooth muscle. *Mol Pharmacol* 59: 692–698, 2001. doi:10.1124/mol.59.4.692.

137. Liang W, Ferrara N. The Complex Role of Neutrophils in Tumor Angiogenesis and Metastasis. *Cancer Immunol Res* 4: 83–91, 2016. doi:10.1158/2326-6066.CIR-15-0313.

138. Lin DC, Bullock CM, Ehlert FJ, Chen JL, Tian H, Zhou QY. Identification and molecular characterization of two closely related G protein-coupled receptors activated by prokineticins/endocrine gland vascular endothelial growth factor. *J Biol Chem* 277: 19276–19280, 2002. doi:10.1074/jbc.M202139200.

139. Lin R, LeCouter J, Kowalski J, Ferrara N. Characterization of endocrine gland-derived vascular endothelial growth factor signaling in adrenal cortex capillary endothelial cells. *J Biol Chem* 277: 8724–8729, 2002. doi:10.1074/jbc.M110594200.

140. Lin WW, Karin M. A cytokine-mediated link between innate immunity, inflammation, and cancer. *J Clin Invest* 117: 1175–1183, 2007. doi:10.1172/JCI31537.

141. Lin X, Kim YA, Lee BL, Söderhäll K, Söderhäll I. Identification and properties of a receptor for the invertebrate cytokine astakine, involved in hematopoiesis. *Exp Cell Res* 315: 1171–1180, 2009. doi:10.1016/j.yexcr.2009.01.001.

142. Lu R, Kujawski M, Pan H, Shively JE. Tumor angiogenesis mediated by myeloid cells is negatively regulated by CEACAM1. *Cancer Res* 72: 2239–2250, 2012. doi:10.1158/0008-5472.CAN-11-3016.

143. Maftei D, Marconi V, Florenzano F, Giancotti LA, Castelli M, Moretti S, Borsani E, Rodella LF, Balboni G, Luongo L, Maione S, Sacerdote P, Negri L, Lattanzi R. Controlling the activation of the Bv8/prokineticin system reduces neuroinflammation and abolishes thermal and tactile hyperalgesia in neuropathic animals. *Br J Pharmacol* 171: 4850–4865, 2014. doi:10.1111/bph.12793.

144. Maldonado-Pérez D, Evans J, Denison F, Millar RP, Jabbour HN. Potential roles of the prokineticins in reproduction. *Trends Endocrinol Metab* 18: 66–72, 2007. doi:10.1016/j.tem.2006.12.002.

145. Mansour HA, Wood J, Logue T, Chowdari KV, Dayal M, Kupfer DJ, Monk TH, Devlin B, Nimgaonkar VL. Association study of eight circadian genes with bipolar I disorder, schizoaffective disorder and schizophrenia. *Genes Brain Behav* 5: 150–157, 2006. doi:10.1111/j.1601-183X.2005.00147.x.

146. Mantovani A, Romero P, Palucka AK, Marincola FM. Tumour immunity: effector response to tumour and role of the microenvironment. *Lancet* 371: 771–783, 2008. doi:10.1016/S0140-6736(08)60241-X.

147. Marigo I, Dolcetti L, Serafini P, Zanovello P, Bronte V. Tumor-induced tolerance and immune suppression by myeloid derived suppressor cells. *Immunol Rev* 222: 162–179, 2008. doi:10.1111/j.1600-065X.2008.00602.x.

148. Marsango S, Bonaccorsi di Patti MC, Barra D, Miele R. Evidence that prokineticin receptor 2 exists as a dimer in vivo. *Cell Mol Life Sci* 68: 2919–2929, 2011. doi:10.1007/s00018-010-0601-6.

149. Martin C, Balasubramanian R, Dwyer AA, Au MG, Sidis Y, Kaiser UB, Seminara SB, Pitteloud N, Zhou QY, Crowley WF Jr. The role of the prokineticin 2 pathway in human reproduction: evidence from the study of human and murine gene mutations. *Endocr Rev* 32: 225–246, 2011. doi:10.1210/er.2010-0007.

150. Martucci C, Franchi S, Giannini E, Tian H, Melchiorri P, Negri L, Sacerdote P. Bv8, the amphibian homologue of the mammalian prokineticins, induces a proinflammatory phenotype of mouse macrophages. *Br J Pharmacol* 147: 225–234, 2006. doi:10.1038/sj.bjp.0706467.

151. Masuda Y, Takatsu Y, Terao Y, Kumano S, Ishibashi Y, Suenaga M, Abe M, Fukusumi S, Watanabe T, Shintani Y, Yamada T, Hinuma S, Inatomi N, Ohtaki T, Onda H, Fujino M. Isolation and identification of EG-VEGF/prokineticins as cognate ligands for two orphan G-protein-coupled receptors. *Biochem Biophys Res Commun* 293: 396–402, 2002. doi:10.1016/S0006-291X(02)00239-5.

152. Matsumoto S, Yamazaki C, Masumoto KH, Nagano M, Naito M, Soga T, Hiyama H, Matsumoto M, Takasaki J, Kamohara M, Matsuo A, Ishii H, Kobori M, Katoh M, Matsushime H, Furuichi K, Shigeyoshi Y. Abnormal development of the olfactory bulb and reproductive system in mice lacking prokineticin receptor PKR2. *Proc Natl Acad Sci USA* 103: 4140–4145, 2006. doi:10.1073/pnas.0508881103.

153. McCabe MJ, Gaston-Massuet C, Gregory LC, Alatzoglou KS, Tziaferi V, Sbai O, Rondard P, Masumoto KH, Nagano M, Shigeyoshi Y, Pfeifer M, Hulse T, Buchanan CR, Pitteloud N, Martinez-Barbera JP, Dattani MT. Variations in PROKR2, but not PROK2, are associated with hypopituitarism and septo-optic dysplasia. *J Clin Endocrinol Metab* 98: E547–E557, 2013. doi:10.1210/jc.2012-3067.

154. McCoach CE, Rogers JG, Dwyre DM, Jonas BA. Paraneoplastic Leukemoid Reaction as a Marker of Tumor Progression in Non-Small Cell Lung Cancer. *Cancer Treat Commun* 4: 15–18, 2015. doi:10.1016/j.ctrc.2015.03.003.

155. Melchiorri D, Bruno V, Besong G, Ngomba RT, Cuomo L, De Blasi A, Copani A, Moschella C, Storto M, Nicoletti F, Lepperdinger G, Passarelli F. The mammalian homologue of the novel peptide Bv8 is expressed in the central nervous system and supports neuronal survival by activating the MAP kinase/PI-3-kinase pathways. *Eur J Neurosci* 13: 1694–1702, 2001. doi:10.1046/j.1460-9568.2001.01549.x.

156. Merrill JA. Ovarian hilus cells. *Am J Obstet Gynecol* 78: 1258–1271, 1959. doi:10.1016/0002-9378(59)90588-5.

157. Miotla J, Maciewicz R, Kendrew J, Feldmann M, Paleolog E. Treatment with soluble VEGF receptor reduces disease severity in murine collagen-induced arthritis. *Lab Invest* 80: 1195–1205, 2000. doi:10.1038/labinvest.3780127.

158. Mollay C, Wechselberger C, Mignogna G, Negri L, Melchiorri P, Barra D, Kreil G. Bv8, a small protein from frog skin and its homologue from snake venom induce hyperalgesia in rats. *Eur J Pharmacol* 374: 189–196, 1999. doi:10.1016/S0014-2999(99)00229-0.

159. Monnier C, Dodé C, Fabre L, Teixeira L, Labesse G, Pin JP, Hardelin JP, Rondard P. PROKR2 missense mutations associated with Kallmann syndrome impair receptor signalling activity. *Hum Mol Genet* 18: 75–81, 2009. doi:10.1093/hmg/ddn318.

160. Monnier J, Quillien V, Piquet-Pellorce C, Leberre C, Preisser L, Gascan H, Samson M. Prokineticin 1 induces CCL4, CXCL1 and CXCL8 in human monocytes but not in macrophages and dendritic cells. *Eur Cytokine Netw* 19: 166–175, 2008.

161. Monnier J, Samson M. Cytokine properties of prokineticins. *FEBS J* 275: 4014–4021, 2008. doi:10.1111/j.1742-4658.2008.06559.x.

162. Nebigil CG. Prokineticin receptors in cardiovascular function: foe or friend? *Trends Cardiovasc Med* 19: 55–60, 2009. doi:10.1016/j.tcm.2009.04.007.

163. Negri L, Lattanzi R. Bv8/PK2 and prokineticin receptors: a druggable pronociceptive system. *Curr Opin Pharmacol* 12: 62–66, 2012. doi:10.1016/j.coph.2011.10.023.

164. Negri L, Lattanzi R, Giannini E, Canestrelli M, Nicotra A, Melchiorri P. Bv8/Prokineticins and their Receptors: A New Pronociceptive System. *Int Rev Neurobiol* 85: 145–157, 2009. doi:10.1016/S0074-7742(09)85011-3.

165. Negri L, Lattanzi R, Giannini E, Colucci M, Margheriti F, Melchiorri P, Vellani V, Tian H, De Felice M, Porreca F. Impaired nociception and inflammatory pain sensation in mice lacking the prokineticin receptor PKR1: focus on interaction between PKR1 and the capsaicin receptor TRPV1 in pain behavior. *J Neurosci* 26: 6716–6727, 2006. doi:10.1523/JNEUROSCI.5403-05.2006.

166. Negri L, Lattanzi R, Giannini E, Colucci MA, Mignogna G, Barra D, Grohovaz F, Codazzi F, Kaiser A, Kreil G, Melchiorri P. Biological activities of Bv8 analogues. *Br J Pharmacol* 146: 625–632, 2005. doi:10.1038/sj.bjp.0706376.

167. Negri L, Lattanzi R, Giannini E, De Felice M, Colucci A, Melchiorri P. Bv8, the amphibian homologue of the mammalian prokineticins, modulates ingestive behaviour in rats. *Br J Pharmacol* 142: 181–191, 2004. doi:10.1038/sj.bjp.0705686.

168. Negri L, Lattanzi R, Giannini E, Melchiorri P. Bv8/Prokineticin proteins and their receptors. *Life Sci* 81: 1103–1116, 2007. doi:10.1016/j.lfs.2007.08.011.

169. Negri L, Lattanzi R, Giannini E, Melchiorri P. Modulators of pain: Bv8 and prokineticins. *Curr Neuropharmacol* 4: 207–215, 2006. doi:10.2174/157015906778019518.

170. Negri L, Lattanzi R, Giannini E, Metere A, Colucci M, Barra D, Kreil G, Melchiorri P. Nociceptive sensitization by the secretory protein Bv8. *Br J Pharmacol* 137: 1147–1154, 2002. doi:10.1038/sj.bjp.0704995.

171. Ng KL, Li JD, Cheng MY, Leslie FM, Lee AG, Zhou QY. Dependence of olfactory bulb neurogenesis on prokineticin 2 signaling. *Science* 308: 1923–1927, 2005. doi:10.1126/science.1112103.

172. Ngan ES, Lee KY, Sit FY, Poon HC, Chan JK, Sham MH, Lui VC, Tam PK. Prokineticin-1 modulates proliferation and differentiation of enteric neural crest cells. *Biochim Biophys Acta* 1773: 536–545, 2007. doi:10.1016/j.bbamcr.2007.01.013.

173. Ngan ES, Lee KY, Yeung WS, Ngan HY, Ng EH, Ho PC. Endocrine gland-derived vascular endothelial growth factor is expressed in human peri-implantation endometrium, but not in endometrial carcinoma. *Endocrinology* 147: 88–95, 2006. doi:10.1210/en.2005-0543.

174. Ngan ES, Sit FY, Lee K, Miao X, Yuan Z, Wang W, Nicholls JM, Wong KK, Garcia-Barcelo M, Lui VC, Tam PK. Implications of endocrine gland-derived vascular endothelial growth factor/prokineticin-1 signaling in human neuroblastoma progression. *Clin Cancer Res* 13: 868–875, 2007. doi:10.1158/1078-0432.CCR-06-2176.

175. Ngan ES, Tam PK. Prokineticin-signaling pathway. *Int J Biochem Cell Biol* 40: 1679–1684, 2008. doi:10.1016/j.biocel.2008.03.010.

176. Pan H, Shively JE. Carcinoembryonic antigen-related cell adhesion molecule-1 regulates granulopoiesis by inhibition of granulocyte colony-stimulating factor receptor. *Immunity* 33: 620–631, 2010. doi:10.1016/j.immuni.2010.10.009.

177. Parker KL, Rice DA, Lala DS, Ikeda Y, Luo X, Wong M, Bakke M, Zhao L, Frigeri C, Hanley NA, Stallings N, Schimmer BP. Steroidogenic factor 1: an essential mediator of endocrine development. *Recent Prog Horm Res* 57: 19–36, 2002. doi:10.1210/rp.57.1.19.

178. Parker R, Liu M, Eyre HJ, Copeland NG, Gilbert DJ, Crawford J, Sutherland GR, Jenkins NA, Herzog H. Y-receptor-like genes GPR72 and GPR73: molecular cloning, genomic organisation and assignment to human chromosome 11q21.1 and 2p14 and mouse chromosome 9 and 6. *Biochim Biophys Acta* 1491: 369–375, 2000. doi:10.1016/S0167-4781(00)00023-3.

179. Pasquali D, Rossi V, Staibano S, De Rosa G, Chieffi P, Prezioso D, Mirone V, Mascolo M, Tramontano D, Bellastella A, Sinisi AA. The endocrine-gland-derived vascular endothelial growth factor (EG-VEGF)/prokineticin 1 and 2 and receptor expression in human prostate: Up-regulation of EG-VEGF/prokineticin 1 with malignancy. *Endocrinology* 147: 4245–4251, 2006. doi:10.1210/en.2006-0614.

180. Peng Z, Tang Y, Luo H, Jiang F, Yang J, Sun L, Li JD. Disease-causing mutation in PKR2 receptor reveals a critical role of positive charges in the second intracellular loop for G-protein coupling and receptor trafficking. *J Biol Chem* 286: 16615–16622, 2011. doi:10.1074/jbc.M111.223784.

181. Phan VT, Wu X, Cheng JH, Sheng RX, Chung AS, Zhuang G, Tran C, Song Q, Kowanetz M, Sambrone A, Tan M, Meng YG, Jackson EL, Peale FV, Junttila MR, Ferrara N. Oncogenic RAS pathway activation promotes resistance to anti-VEGF therapy through G-CSF-induced neutrophil recruitment. *Proc Natl Acad Sci USA* 110: 6079–6084, 2013. doi:10.1073/pnas.1303302110.

182. Pitteloud N, Quinton R, Pearce S, Raivio T, Acierno J, Dwyer A, Plummer L, Hughes V, Seminara S, Cheng YZ, Li WP, Maccoll G, Eliseenkova AV, Olsen SK, Ibrahimi OA, Hayes FJ, Boepple P, Hall JE, Bouloux P, Mohammadi M, Crowley W Jr. Digenic mutations account for variable phenotypes in idiopathic hypogonadotropic hypogonadism. *J Clin Invest* 117: 457–463, 2007. doi:10.1172/JCI29884.

183. Pitteloud N, Zhang C, Pignatelli D, Li JD, Raivio T, Cole LW, Plummer L, Jacobson-Dickman EE, Mellon PL, Zhou QY, Crowley WF Jr. Loss-of-function mutation in the prokineticin 2 gene causes Kallmann syndrome and normosmic idiopathic hypogonadotropic hypogonadism. *Proc Natl Acad Sci USA* 104: 17447–17452, 2007. doi:10.1073/pnas.0707173104.

184. Prosser HM, Bradley A, Chesham JE, Ebling FJ, Hastings MH, Maywood ES. Prokineticin receptor 2 (Prokr2) is essential for the regulation of circadian behavior by the suprachiasmatic nuclei. *Proc Natl Acad Sci USA* 104: 648–653, 2007. doi:10.1073/pnas.0606884104.

185. Qiu CY, Liu YQ, Qiu F, Wu J, Zhou QY, Hu WP. Prokineticin 2 potentiates acid-sensing ion channel activity in rat dorsal root ganglion neurons. *J Neuroinflammation* 9: 108, 2012. doi:10.1186/1742-2094-9-108.

186. Qu X, Zhuang G, Yu L, Meng G, Ferrara N. Induction of Bv8 expression by granulocyte colony-stimulating factor in CD11b+Gr1+ cells: key role of Stat3 signaling. *J Biol Chem* 287: 19574–19584, 2012. doi:10.1074/jbc.M111.326801.

187. Raivio T, Avbelj M, McCabe MJ, Romero CJ, Dwyer AA, Tommiska J, Sykiotis GP, Gregory LC, Diaczok D, Tziaferi V, Elting MW, Padidela R, Plummer L, Martin C, Feng B, Zhang C, Zhou QY, Chen H, Mohammadi M, Quinton R, Sidis Y, Radovick S, Dattani MT, Pitteloud N. Genetic overlap in Kallmann syndrome, combined pituitary hormone deficiency, and septo-optic dysplasia. *J Clin Endocrinol Metab* 97: E694–E699, 2012. doi:10.1210/jc.2011-2938.

188. Ren C, Qiu CY, Gan X, Liu TT, Qu ZW, Rao Z, Hu WP. Prokineticin 2 facilitates mechanical allodynia induced by α,β-methylene ATP in rats. *Eur J Pharmacol* 767: 24–29, 2015. doi:10.1016/j.ejphar.2015.09.047.

189. Ren P, Zhang H, Qiu F, Liu YQ, Gu H, O'Dowd DK, Zhou QY, Hu WP. Prokineticin 2 regulates the electrical activity of rat suprachiasmatic nuclei neurons. *PLoS One* 6: e20263, 2011. doi:10.1371/journal.pone.0020263.

190. Reynaud R, Jayakody SA, Monnier C, Saveanu A, Bouligand J, Guedj AM, Simonin G, Lecomte P, Barlier A, Rondard P, Martinez-Barbera JP, Guiochon-Mantel A, Brue T. PROKR2 variants in multiple hypopituitarism with pituitary stalk interruption. *J Clin Endocrinol Metab* 97: E1068–E1073, 2012. doi:10.1210/jc.2011-3056.

191. Risau W, Flamme I. Vasculogenesis. *Annu Rev Cell Dev Biol* 11: 73–91, 1995. doi:10.1146/annurev.cb.11.110195.000445.

192. Roberts AW. G-CSF: a key regulator of neutrophil production, but that's not all! *Growth Factors* 23: 33–41, 2005. doi:10.1080/08977190500055836.

193. Ruiz-Ferrer M, Torroglosa A, Núñez-Torres R, de Agustín JC, Antiñolo G, Borrego S. Expression of PROKR1 and PROKR2 in human enteric neural precursor cells and identification of sequence variants suggest a role in HSCR. *PLoS One* 6: e23475, 2011. doi:10.1371/journal.pone.0023475.

194. Samson M, Peale FV Jr, Frantz G, Rioux-Leclercq N, Rajpert-De Meyts E, Ferrara N. Human endocrine gland-derived vascular endothelial growth factor: expression early in development and in Leydig cell tumors suggests roles in normal and pathological testis angiogenesis. *J Clin Endocrinol Metab* 89: 4078–4088, 2004. doi:10.1210/jc.2003-032024.

195. Sarfati J, Fouveaut C, Leroy C, Jeanpierre M, Hardelin JP, Dodé C. Greater prevalence of PROKR2 mutations in Kallmann syndrome patients from the Maghreb than in European patients. *Eur J Endocrinol* 169: 805–809, 2013. doi:10.1530/EJE-13-0419.

196. Sarfati J, Guiochon-Mantel A, Rondard P, Arnulf I, Garcia-Piñero A, Wolczynski S, Brailly-Tabard S, Bidet M, Ramos-Arroyo M, Mathieu M, Lienhardt-Roussie A, Morgan G, Turki Z, Bremont C, Lespinasse J, Du Boullay H, Chabbert-Buffet N, Jacquemont S, Reach G, De Talence N, Tonella P, Conrad B, Despert F, Delobel B, Brue T, Bouvattier C, Cabrol S, Pugeat M, Murat A, Bouchard P, Hardelin JP, Dodé C, Young J. A comparative phenotypic study of kallmann syndrome patients carrying monoallelic and biallelic mutations in the prokineticin 2 or prokineticin receptor 2 genes. *J Clin Endocrinol Metab* 95: 659–669, 2010. doi:10.1210/jc.2009-0843.

197. Sbai O, Monnier C, Dodé C, Pin JP, Hardelin JP, Rondard P. Biased signaling through G-protein-coupled PROKR2 receptors harboring missense mutations. *FASEB J* 28: 3734–3744, 2014. doi:10.1096/fj.13-243402.

198. Schweitz H, Pacaud P, Diochot S, Moinier D, Lazdunski M. MIT(1), a black mamba toxin with a new and highly potent activity on intestinal contraction. *FEBS Lett* 461: 183–188, 1999. doi:10.1016/S0014-5793(99)01459-3.

199. Schweizerhof M, Stösser S, Kurejova M, Njoo C, Gangadharan V, Agarwal N, Schmelz M, Bali KK, Michalski CW, Brugger S, Dickenson A, Simone DA, Kuner R. Hematopoietic colony-stimulating factors mediate tumor-nerve interactions and bone cancer pain. *Nat Med* 15: 802–807, 2009. doi:10.1038/nm.1976.

200. Serafini P, Borrello I, Bronte V. Myeloid suppressor cells in cancer: recruitment, phenotype, properties, and mechanisms of immune suppression. *Semin Cancer Biol* 16: 53–65, 2006. doi:10.1016/j.semcancer.2005.07.005.

201. Severini C, Lattanzi R, Maftei D, Marconi V, Ciotti MT, Petrocchi Passeri P, Florenzano F, Del Duca E, Caioli S, Zona C, Balboni G, Salvadori S, Nisticò R, Negri L. Bv8/prokineticin 2 is involved in Aβ-induced neurotoxicity. *Sci Rep* 5: 15301, 2015. doi:10.1038/srep15301.

202. Sher D, Knebel A, Bsor T, Nesher N, Tal T, Morgenstern D, Cohen E, Fishman Y, Zlotkin E. Toxic polypeptides of the hydra–a bioinformatic approach to cnidarian allomones. *Toxicon* 45: 865–879, 2005. doi:10.1016/j.toxicon.2005.02.004.

203. Shojaei F, Ferrara N. Role of the microenvironment in tumor growth and in refractoriness/resistance to anti-angiogenic therapies. *Drug Resist Updat* 11: 219–230, 2008. doi:10.1016/j.drup.2008.09.001.

204. Shojaei F, Singh M, Thompson JD, Ferrara N. Role of Bv8 in neutrophil-dependent angiogenesis in a transgenic model of cancer progression. *Proc Natl Acad Sci USA* 105: 2640–2645, 2008. doi:10.1073/pnas.0712185105.

205. Shojaei F, Wu X, Malik AK, Zhong C, Baldwin ME, Schanz S, Fuh G, Gerber HP, Ferrara N. Tumor refractoriness to anti-VEGF treatment is mediated by CD11b+Gr1+ myeloid cells. *Nat Biotechnol* 25: 911–920, 2007. doi:10.1038/nbt1323.

206. Shojaei F, Wu X, Qu X, Kowanetz M, Yu L, Tan M, Meng YG, Ferrara N. G-CSF-initiated myeloid cell mobilization and angiogenesis mediate tumor refractoriness to anti-VEGF therapy in mouse models. *Proc Natl Acad Sci USA* 106: 6742–6747, 2009. doi:10.1073/pnas.0902280106.

207. Shojaei F, Wu X, Zhong C, Yu L, Liang XH, Yao J, Blanchard D, Bais C, Peale FV, van Bruggen N, Ho C, Ross J, Tan M, Carano RA, Meng YG, Ferrara N. Bv8 regulates myeloid-cell-dependent tumour angiogenesis. *Nature* 450: 825–831, 2007. doi:10.1038/nature06348.

208. Shojaei F, Zhong C, Wu X, Yu L, Ferrara N. Role of myeloid cells in tumor angiogenesis and growth. *Trends Cell Biol* 18: 372–378, 2008. doi:10.1016/j.tcb.2008.06.003.

209. Sinisi AA, Asci R, Bellastella G, Maione L, Esposito D, Elefante A, De Bellis A, Bellastella A, Iolascon A. Homozygous mutation in the prokineticin-receptor2 gene (Val274Asp) presenting as reversible Kallmann syndrome and persistent oligozoospermia: case report. *Hum Reprod* 23: 2380–2384, 2008. doi:10.1093/humrep/den247.

210. Söderhäll I, Kim YA, Jiravanichpaisal P, Lee SY, Söderhäll K. An ancient role for a prokineticin domain in invertebrate hematopoiesis. *J Immunol* 174: 6153–6160, 2005. doi:10.4049/jimmunol.174.10.6153.

211. Soga T, Matsumoto S, Oda T, Saito T, Hiyama H, Takasaki J, Kamohara M, Ohishi T, Matsushime H, Furuichi K. Molecular cloning and characterization of prokineticin receptors. *Biochim Biophys Acta* 1579: 173–179, 2002. doi:10.1016/S0167-4781(02)00546-8.

212. Song J, Li J, Liu HD, Liu W, Feng Y, Zhou XT, Li JD. Snapin interacts with G-protein coupled receptor PKR2. *Biochem Biophys Res Commun* 469: 501–506, 2016. doi:10.1016/j.bbrc.2015.12.023.

213. Sposini S, Caltabiano G, Hanyaloglu AC, Miele R. Identification of transmembrane domains that regulate spatial arrangements and activity of prokineticin receptor 2 dimers. *Mol Cell Endocrinol* 399: 362–372, 2015. doi:10.1016/j.mce.2014.10.024.

214. Springael JY, Urizar E, Parmentier M. Dimerization of chemokine receptors and its functional consequences. *Cytokine Growth Factor Rev* 16: 611–623, 2005. doi:10.1016/j.cytogfr.2005.05.005.

215. Steinman L. Immunology of relapse and remission in multiple sclerosis. *Annu Rev Immunol* 32: 257–281, 2014. doi:10.1146/annurev-immunol-032713-120227.

216. Sternberg WH. The morphology, androgenic function, hyperplasia, and tumors of the human ovarian hilus cells. *Am J Pathol* 25: 493–521, 1949.

217. Strauss JF, Barbieri RL. *Yen & Jaffe's Reproductive Endocrinology* (7th ed.). Philadelphia, PA: Saunders, 2013.

218. Strydom DJ. Snake venom toxins. Purification and properties of low-molecular-weight polypeptides of *Dendroaspis polylepis polylepis* (black mamba) venom. *Eur J Biochem* 69: 169–176, 1976. doi:10.1111/j.1432-1033.1976.tb10870.x.

219. Su MT, Lin SH, Chen YC, Kuo PL. Gene-gene interactions and risk of recurrent miscarriages in carriers of endocrine gland-derived vascular endothelial growth factor and prokineticin receptor polymorphisms. *Fertil Steril* 102: 1071–1077.e3, 2014. doi:10.1016/j.fertnstert.2014.06.042.

220. Sykiotis GP, Plummer L, Hughes VA, Au M, Durrani S, Nayak-Young S, Dwyer AA, Quinton R, Hall JE, Gusella JF, Seminara SB, Crowley WF Jr, Pitteloud N. Oligogenic basis of isolated gonadotropin-releasing hormone deficiency. *Proc Natl Acad Sci USA* 107: 15140–15144, 2010. doi:10.1073/pnas.1009622107.

221. Szatkowski C, Vallet J, Dormishian M, Messaddeq N, Valet P, Boulberdaa M, Metzger D, Chambon P, Nebigil CG. Prokineticin receptor 1 as a novel suppressor of preadipocyte proliferation and differentiation to control obesity. *PLoS One* 8: e81175, 2013. doi:10.1371/journal.pone.0081175.

222. Szekanecz Z, Besenyei T, Paragh G, Koch AE. Angiogenesis in rheumatoid arthritis. *Autoimmunity* 42: 563–573, 2009. doi:10.1080/08916930903143083.

223. Szeto TH, Wang XH, Smith R, Connor M, Christie MJ, Nicholson GM, King GF. Isolation of a funnel-web spider polypeptide with homology to mamba intestinal toxin 1 and the embryonic head inducer Dickkopf-1. *Toxicon* 38: 429–442, 2000. doi:10.1016/S0041-0101(99)00174-9.

224. Tabata S, Goi T, Nakazawa T, Kimura Y, Katayama K, Yamaguchi A. Endocrine gland-derived vascular endothelial growth factor strengthens cell invasion ability via prokineticin receptor 2 in colon cancer cell lines. *Oncol Rep* 29: 459–463, 2013. doi:10.3892/or.2012.2124.

225. Tan PH, Yang LC, Shih HC, Lan KC, Cheng JT. Gene knockdown with intrathecal siRNA of NMDA receptor NR2B subunit reduces formalin-induced nociception in the rat. *Gene Ther* 12: 59–66, 2005. doi:10.1038/sj.gt.3302376.

226. Tiberi F, Tropea A, Romani F, Apa R, Marana R, Lanzone A. Prokineticin 1, homeobox A10, and progesterone receptor messenger ribonucleic acid expression in primary cultures of endometrial stromal cells isolated from endometrium of healthy women and from eutopic endometrium of women with endometriosis. *Fertil Steril* 94: 2558–2563, 2010. doi:10.1016/j.fertnstert.2010.03.006.

227. Tommiska J, Toppari J, Vaaralahti K, Känsäkoski J, Laitinen EM, Noisa P, Kinnala A, Niinikoski H, Raivio T. PROKR2 mutations in autosomal recessive Kallmann syndrome. *Fertil Steril* 99: 815–818, 2013. doi:10.1016/j.fertnstert.2012.11.003.

228. Tsuda M, Kohro Y, Yano T, Tsujikawa T, Kitano J, Tozaki-Saitoh H, Koyanagi S, Ohdo S, Ji RR, Salter MW, Inoue K. JAK-STAT3 pathway regulates spinal astrocyte proliferation and neuropathic pain maintenance in rats. *Brain* 134: 1127–1139, 2011. doi:10.1093/brain/awr025.

229. Urayama K, Dedeoglu DB, Guilini C, Frantz S, Ertl G, Messaddeq N, Nebigil CG. Transgenic myocardial overexpression of prokineticin receptor-2 (GPR73b) induces hypertrophy and capillary vessel leakage. *Cardiovasc Res* 81: 28–37, 2009. doi:10.1093/cvr/cvn251.

230. Urayama K, Guilini C, Messaddeq N, Hu K, Steenman M, Kurose H, Ert G, Nebigil CG. The prokineticin receptor-1 (GPR73) promotes cardiomyocyte survival and angiogenesis. *FASEB J* 21: 2980–2993, 2007. doi:10.1096/fj.07-8116com.

231. Urayama K, Guilini C, Turkeri G, Takir S, Kurose H, Messaddeq N, Dierich A, Nebigil CG. Prokineticin receptor-1 induces neovascularization and epicardial-derived progenitor cell differentiation. *Arterioscler Thromb Vasc Biol* 28: 841–849, 2008. doi:10.1161/ATVBAHA.108.162404.

232. Vellani V, Colucci M, Lattanzi R, Giannini E, Negri L, Melchiorri P, McNaughton PA. Sensitization of transient receptor potential vanilloid 1 by the prokineticin receptor agonist Bv8. *J Neurosci* 26: 5109–5116, 2006. doi:10.1523/JNEUROSCI.3870-05.2006.

233. Von Hunolstein JJ, Nebigil CG. Can prokineticin prevent obesity and insulin resistance? *Curr Opin Endocrinol Diabetes Obes* 22: 367–373, 2015. doi:10.1097/MED.0000000000000185.

234. Wade PR, Palmer JM, Mabus J, Saunders PR, Prouty S, Chevalier K, Gareau MG, McKenney S, Hornby PJ. Prokineticin-1 evokes secretory and contractile activity in rat small intestine. *Neurogastroenterol Motil* 22: e152–e161, 2010.

235. Wang CY, Tsai HL, Syu JS, Chen TY, Su MT. Primary Cilium-Regulated EG-VEGF Signaling Facilitates Trophoblast Invasion. *J Cell Physiol* 232: 1467–1477, 2017. doi:10.1002/jcp.25649.

236. Wang Y, Guo X, Ma H, Lu L, Zhang R. Prokineticin-2 is associated with metabolic syndrome in a middle-aged and elderly Chinese population. *Lipids Health Dis* 15: 1, 2016. doi:10.1186/s12944-015-0172-5.

237. Webb EA, Dattani MT. Septo-optic dysplasia. *Eur J Hum Genet* 18: 393–397, 2010. doi:10.1038/ejhg.2009.125.

238. Wechselberger C, Puglisi R, Engel E, Lepperdinger G, Boitani C, Kreil G. The mammalian homologues of frog Bv8 are mainly expressed in spermatocytes. *FEBS Lett* 462: 177–181, 1999. doi:10.1016/S0014-5793(99)01473-8.

239. Wen S, Wilson DT, Kuruppu S, Korsinczky ML, Hedrick J, Pang L, Szeto T, Hodgson WC, Alewood PF, Nicholson GM. Discovery of an MIT-like atracotoxin family: spider venom peptides that share sequence homology but not pharmacological properties with AVIT family proteins. *Peptides* 26: 2412–2426, 2005. doi:10.1016/j.peptides.2005.05.012.

240. Xin H, Lu R, Lee H, Zhang W, Zhang C, Deng J, Liu Y, Shen S, Wagner KU, Forman S, Jove R, Yu H. G-protein-coupled receptor agonist BV8/prokineticin-2 and STAT3 protein form a feed-forward loop in both normal and malignant myeloid cells. *J Biol Chem* 288: 13842–13849, 2013. doi:10.1074/jbc.M113.450049.

241. Xiong YC, Li XM, Wang XJ, Liu YQ, Qiu F, Wu D, Gan YB, Wang BH, Hu WP. Prokineticin 2 suppresses GABA-activated current in rat primary sensory neurons. *Neuropharmacology* 59: 589–594, 2010. doi:10.1016/j.neuropharm.2010.08.014.

242. Yan B, Wei JJ, Yuan Y, Sun R, Li D, Luo J, Liao SJ, Zhou YH, Shu Y, Wang Q, Zhang GM, Feng ZH. IL-6 cooperates with G-CSF to induce protumor function of neutrophils in bone marrow by enhancing STAT3 activation. *J Immunol* 190: 5882–5893, 2013. doi:10.4049/jimmunol.1201881.

243. Yang L, DeBusk LM, Fukuda K, Fingleton B, Green-Jarvis B, Shyr Y, Matrisian LM, Carbone DP, Lin PC. Expansion of myeloid immune suppressor Gr+CD11b+ cells in tumor-bearing host directly promotes tumor angiogenesis. *Cancer Cell* 6: 409–421, 2004. doi:10.1016/j.ccr.2004.08.031.

244. Yin W, Liu H, Peng Z, Chen D, Li J, Li JD. Mechanisms that underlie the internalization and extracellular signal regulated kinase 1/2 activation by PKR2 receptor. *Cell Signal* 26: 1118–1124, 2014. doi:10.1016/j.cellsig.2014.01.031.

245. Yuill EA, Hoyda TD, Ferri CC, Zhou QY, Ferguson AV. Prokineticin 2 depolarizes paraventricular nucleus magnocellular and parvocellular neurons. *Eur J Neurosci* 25: 425–434, 2007. doi:10.1111/j.1460-9568.2006.05293.x.

246. Zeleznik AJ. Follicle selection in primates: "many are called but few are chosen". *Biol Reprod* 65: 655–659, 2001. doi:10.1095/biolreprod65.3.655.

247. Zhang C, Ng KL, Li JD, He F, Anderson DJ, Sun YE, Zhou QY. Prokineticin 2 is a target gene of proneural basic helix-loop-helix factors for olfactory bulb neurogenesis. *J Biol Chem* 282: 6917–6921, 2007. doi:10.1074/jbc.C600290200.

248. Zhang C, Truong KK, Zhou QY. Efferent projections of prokineticin 2 expressing neurons in the mouse suprachiasmatic nucleus. *PLoS One* 4: e7151, 2009. doi:10.1371/journal.pone.0007151.

249. Zhang L, Yang N, Conejo-Garcia JR, Katsaros D, Mohamed-Hadley A, Fracchioli S, Schlienger K, Toll A, Levine B, Rubin SC, Coukos G. Expression of endocrine gland-derived vascular endothelial growth factor in ovarian carcinoma. *Clin Cancer Res* 9: 264–272, 2003.

250. Zhong C, Qu X, Tan M, Meng YG, Ferrara N. Characterization and regulation of bv8 in human blood cells. *Clin Cancer Res* 15: 2675–2684, 2009. doi:10.1158/1078-0432.CCR-08-1954.

251. Zhou QY. The prokineticins: a novel pair of regulatory peptides. *Mol Interv* 6: 330–338, 2006. doi:10.1124/mi.6.6.6.

252. Zhou QY, Cheng MY. Prokineticin 2 and circadian clock output. *FEBS J* 272: 5703–5709, 2005. doi:10.1111/j.1742-4658.2005.04984.x.

253. Zhou W, Li JD, Hu WP, Cheng MY, Zhou QY. Prokineticin 2 is involved in the thermoregulation and energy expenditure. *Regul Pept* 179: 84–90, 2012. doi:10.1016/j.regpep.2012.08.003.

254. Zhou XT, Chen DN, Xie ZQ, Peng Z, Xia KD, Liu HD, Liu W, Su B, Li JD. Functional analysis of the distal region of the third intracellular loop of PROKR2. *Biochem Biophys Res Commun* 439: 12–17, 2013. doi:10.1016/j.bbrc.2013.08.039.