Helicobacter pylori cytotoxin: A novel ligand for receptor-like protein tyrosine phosphatase ß (Review)

TOSHIO INUI[1], SHIGETO MIZUNO[2], KENJI TAKAI[1], MASAO NAKAGAWA[3], MATSUMI UCHIDA[4], MINEKO FUJIMIYA[5], AKIHIRO ASAKAWA[6] and AKIO INUI[6]

[1]Inui Clinic, Osaka; [2]Department of Gastroenterology, Kinki University Shool of Medicine, Osaka; [3]Second Department of Internal Medicine, Kyoto Prefectural University of Medicine and Nakagawa Clinic, Kyoto; [4]Department of Urology, Kyoto Prefectural University of Medicine and Matsushita Memorial Hospital, Kyoto; [5]Department of Anatomy, Shiga University of Medical Science, Shiga; [6]Division of Diabetes, Digestive and Kidney Diseases, Department of Clinical Molecular Medicine, Kobe University Graduate School of Medicine, Kobe, Japan

Received March 21, 2003; Accepted August 25, 2003

Abstract. The impacts of toxins are generally observed as acute intoxications, whereas the environmental health effects of chronic exposure to mostly low levels of toxins are only poorly documented and are an emerging issue. Furthermore, toxins are potentially useful as tools to study the physiological role of particular channels or to determine structural features of channels that are important for their function. Recent studies suggest that vacuolating cytotoxin (VacA) is a novel ligand for receptor protein tyrosine phosphatase (RPTP) ß. VacA is a virulence factor of *Helicobacter pylori* that is involved in gastric ulceration and cancer in experimental animals and humans. VacA may represent the chronic exposure of A-B family of bacterial endotoxins and be a useful probe for RPTPß functions in the gastrointestinal system.

Contents

1. Introduction
2. Vacuolating cytotoxin (VacA)
3. VacA interacts with full-length receptor protein tyrosine phosphatase ß
4. Novel ligand for RPTPß
5. Toxins as tools for channel and receptor functions
6. Concluding remarks

Correspondence to: Dr Akio Inui, Division of Diabetes, Digestive and Kidney Diseases, Department of Clinical Molecular Medicine, Kobe University Graduate School of Medicine, 7-5-1 Kusunoki-cho, Chuo-ku, Kobe 650-0017, Japan
E-mail: inui@med.kobe-u.ac.jp

Key words: Helicobacter pylori, tyrosine phosphatase ß

1. Introduction

Helicobacter pylori is a spiral-shaped Gram-negative bacterium endowed with a very powerful urease activity and with polar flagella (1). These features allow the bacterium to survive at acidic pH in the stomach lumen. *H. pylori* is probably most common chronic bacterium infection of humans, present in almost half of the world population. Once acquired, these organisms persist for years, for decades, and possibly for life. The presence of the bacterium in the gastric mucosa is associated with chronic active gastritis and is implicated in more severe gastric diseases, including peptic ulceration, mucosa-associated lymphoid tissue lymphoma, and gastric carcinoma (1,2).

2. Vacuolating cytotoxin (VacA)

Many *H. pylori* strains secrete a cytotoxin that collective evidence indicates is an important virulence factor in *H. pylori*-mediated disease (3,4). The toxin was named vacuolating cytotoxin (VacA) because it induces vacuolation of cultured mammalian cells. The VacA gene has a 3864- or 4200-base pair open reading frame, encoding a precursor protein for the mature 87- or 95-kDa VacA. Oral administration of purified VacA to mice induces gastric mucosa degeneration and inflammatory cell recruitment, characteristic of *H. pylori*-mediated diseases (5).

VacA exhibits cellular behavior similar to that of protein toxin with intracellular targets. Most intracellularly acting toxins posess an overall structure corresponding to the A-B family of bacterial endotoxins, which includes diphtheria, cholera, and anthrax toxins (4,6). In general, the B component of an A-B toxin binds to a specific mammalian cell-surface receptor and facilitates the membrane translocation of an enzyme A moiety into the cytosol. The A moiety covalently modifies a specific intracellular target molecule, resulting in the disruption of important cellular functions. VacA has recently been indicated to be an A-B toxin and to bind to specific, high affinity cell surface receptors (7,8).

The formation of the vaculoles is accompanied by a redistribution of lysosomal membrane glycoproteins among endocytic compartments. The membranes of the vacuoles contain the small GTP-binding protein Rab7 (a late endosomal marker) and the membrane glycoprotein Lgp 110 (a lysosomal marker), suggesting that VacA may disrupt normal membrane trafficking at or near the level of late endosomes (9). The function of Rab7 is required for vacuole formation since HeLa cells overexpressing dominant negative mutants of Rab7 do not develop vacuoles upon exposure to VacA (10). Dynamin is a large-molecular weight GTP-binding protein which is required for intracellular vesicle transportation (11-13). A recent study demonstrated that VacA failed to induce vacuolation in HeLa cells transfected with dominant-negative dynamin and that stable transfection of wild-type dynamin augmented VacA-induced vacuolation, indicating a crucial role of dynamin in VacA-induced vacuolation (14). A protein (VIP54) co-localizing with the intermediate filament protein vimentin was recently found to be a possible cytosolic target of VacA (15). The association of intermediate filament proteins with the nuclear membrane and with the plasmelemma is well documented although the role of VacA-VIP54-vimentin in vacuole biogenesis needs to be clarified (15). Vacuole formation is also dependent on the function of a vacuolar-type ATPase protein pump in transfected cells as well as in response to externally added VacA (3,9). Late endosomes may represent an important crossroad involved in the transport of endocytosed ligands and newly synthesized proteins that are destined for lysosomes (9). Any pertubation of cellular function at this level may have important functional consequences. The demonstration that VacA inhibits intracellular degradation of epidermal growth factor (EGF) and inhibits normal maturation of procathepsin D may illustrate two important functional alterations (4,16).

VacA applied extracellularly apparently targets mitochondria (17). Such a translocation could be accomplished in an endosomal compartment, by a mechanism similar to those described for other A-B toxins (18). VacA released by *H. pylori* is often cleaved into two subunits of 34 kDa (the N-terminal fragment, p34) and 58 kDa (the C-terminal fragment, p58), which remain associated by non-covalant interactions (3,19). The toxin is able to bind a cell receptor through a segment of the p58 domain (7). Upon binding, VacA could either be endocytosed and accumulated in late endosomes where vacuoles originate, or translocated into the cytosol where it could exert its action (17). VacA p34 is targeted selectively to mitochondria where it induces cytochrome c release and apoptosis (17). Several other bacterial toxins have been shown to induce mitochondrial dysfunction, including *clostridium difficile* toxin A (20), *Escherichia coli* lipopolysaccharide (21), and cereulide, an emetic toxin isolated from *Bacillus cereus* (22). It was also found that the minimal intracellular vacuolating domain of VacA encompasses p34 and <200 residues belonging to the C-terminal moiety of p58 (6).

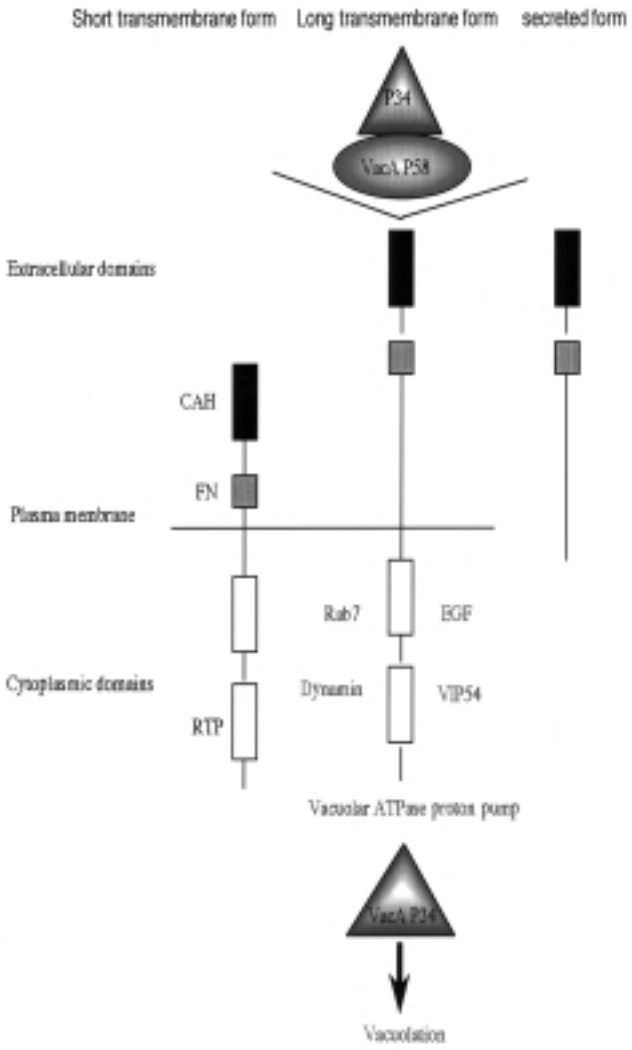

Figure 1. Receptor-like protein tyrosine phasphatase ß (RPTPß) family. A wide variety of stimuli, including growth factors, cytokines, hormones, extracellular matrix components, and cell adhesion molecules, transmit signals via pathways involving tyrosyl phosphorylation of specific cellular proteins. Phosphorylation of a particular site is determined by the balance between phosphatase addition by protein kinases and its removal by phosphatases. RPTPs have a single membrane-spanning domain, and most contain tandem intracellular PTP domains, with the one closer to the membrane typically having greater or sometimes all the catalytic activity (25). RPTPß is unique in the PTP family in that three forms are generated by alternative splicing: a long and short transmembrane form, both of which contain the catalytic domains, and a secreted form of the protein (phosphacan), which lacks the transmembrane and intracellular portions. All RPTPß isoforms contain a carbonic anhydrase domain (CAH), a fibronection type III repeat (FN) and a spacer domain. The long receptor form and the secreted form have a large extracellular insert rich in glycosaminoglycan side chains. *Helicobacter pylori* secretes a potent cytotoxin (VacA), which induces cytoplasmic vacuolation in some eukaryotic cells. VacA is able to interact with target cells by binding to a long receptor form of RPTPß (23), probably through the p58 domain. The cellular targets by which VacA induces vacuolation include Rab7 (10), dynamin (14), epidermal growth factor (EGF) receptor (8), VIP54 (15), and vacuolar ATPase protein pump (3,9). VacA p34 is targeted to mitochondria where it induces dys-function and apoptosis. The mechanisms by which vacuolation is related to signaling from RPTPß to other molecules in the stomach need to be further examined.

3. VacA interacts with full-length receptor protein tyrosine phosphatase ß

It was recently reported that VacA is able to interact with target cells by binding to a 250-kDa receptor protein tyrosine phosphatase (RPTP)ß (23,24). VacA is known to cause vacuolation in many cell types, with the notable exception of HeLa-60. Even in this cell, VacA sensitivity clearly paralleled the induction of RPTPß mRNA expression by

phorbor 12-myristate 13-acetate (PMA) (24). Transfection of RPTPß gene into the hamster kidney cells that are also insensitive to VacA, resulted in an induction of VacA sensitivity (24). RPTPß is a receptor-like protein tyrosine phosphatase composed on an extracellular domain, a single transmembrane domain and a cytoplasmic portion that contains two tyrosine phosphatase domains (25,26) (Fig. 1). Three different isoforms of RPTPß are expressed as a result of alternative splicing: a short and a long form (transmembrane) that differ by the presence of the spacer region of the extracellular domain and a secreted form lacking phosphatase activity. VacA was demonstrated to bind to the full-length 250-kDa isoform (23). Many agents induce cellular responses by regulating the tyrosine phosphorylation of target proteins including the EGF receptor (25,26). It is known that EGF-activated signal transduction pathways are essential for cell proliferation and ulcer healing, and vacuolation induced by VacA is inhibited by an antibody against EGF receptor (8).

4. Novel ligand for RPTPß

The extracellular region of RPTPß has a complex structure, with multiple domains that may interact with a variety of ligands. Such ligands include the extracellular matrix protein tenascin, pleiotrophin, and several neuronal cell adhesion molecules (CAMs) from the immunoglobulin superfamily, which are important for the establishment of intricate networks of connections in the nervous system (25,26). Recently pleiotrophin was demonstrated to be the first natural ligand for transmembrane forms of RPTPß as well as for any of the RPTP family (27). Pleiotrophin is a platelet-derived growth factor-inducible, 18-kDa heparin binding cytokine that signals diverse phenotypes in normal and dysregulated cellular growth and differentiation. Pleiotrophin may signal through ligand-dependent receptor inactivation of RPTPß to increase levels of tyrosine phosphorylation of ß catenin to initiate downstream signaling. It may be important in cell adhesion, tumor invasiveness, and metastasis (27).

RPTPß isoforms are found in the developing nervous system primarily on radial glia and astrocytes, in patterns suggestive of their involvement in neuronal migration and axonal guidance (25). The isoforms, however, differ in developmental pattern of expression and abundance in different brain regions. It appears that the short form present on the surface of glial cells promotes neurite outgrowth by interacting with certain CAMs, while the long or the secreted form can have either inhibitory or repulsive effects (25,28). The transmembrane forms of RPTPß can be pharmacologically discriminated from each other by their different sensitivities to VacA. Mapping the receptor binding as well as other functional domains will be important for understanding the mechanism of VacA cytotoxicity, and also useful as a biochemical probe for the long receptor form of RPTPß.

5. Toxins as tools for channel and receptor functions

Neurons of the mammalian brain express multiple subtypes of sodium channels that are the products of at least six distinct genes (29-33). These channels differ in subcellular localization, developmental pattern of expression, and abundance in different brain regions. These variations are suggestive of functional differences, yet there is no direct information about different physiological roles of particular sodium channel subtypes. Several scorpion toxins have been shown to exert their neurotoxic effects by a direct interaction with voltage-dependent sodium channels (30,31). Recent studies suggest that multiple sodium channel subtypes in mammalian brain can be pharmacologically discriminated by their sensitivity to certain toxins, such as scorpion α-toxins and α-like toxins (32,34). Sodium channel specific scorpion toxins are peptides of 60-76 amino acid residues in length, tightly bound by four disulfide bridge (35). The complete amino acid sequence of 85 distinct peptides are presently known (35). Interestingly, sodium channels have been demonstrated to interact with RPTPß, which increases sodium currents (36).

Calcium channels play key roles in numerous physiological processes including neurotransmitter release, hormone secretion, neurite outgrowth, and gene expression (37,38). Neurons express several distinct types of voltage-gated calcium channels, which fall into two main categories: high voltage-activated and low voltage-activated channels. High voltage-activated channels are classified as L-, N-, P/Q-, and R-types, based primarily on pharmacological criteria (39,40). The N-type channels are uniquely sensitive to spider toxin ω-conotoxin GV1A and M VIIA, and P-type channels to low concentrations of ω-agatoxin-1VA (37,38,41). Another spider toxin ω-agatoxin IIIA may define a unique, high affinity binding sites on N-, P/Q-, and R-type calcium channels (41).

Potassium channels represent a large and diverse collection of membrane proteins whose common property is that they allow the permeation of potassium with high selectivity (42). Potassium channels participate in a large number of important cellular functions such as control of cell electrical excitability and excitation/response coupling. Various inhibitors of potassium channels were obtained from a variety of sources (42-45). Peptides from scorpions were found to block either of two major classes of K^+ channels, voltage-gated (Kv-type) and high conductance Ca^{2+}-activated (BK-type) K^+ channels (43). Other examples include the muscarinic and nicotinic acetylcholine receptors. Muscarinic acetylcholine receptors exist as five subtypes that are widely distributed throughout the body. They are involved in the regulation of fundamental physiological processes, like the modulation of the heart rate, control of motor system and modulation of learning and memory (46). Conventional pharmacological agents were not highly selective for particular subtypes, making investigations on the functional significance of the subtypes difficult (46,47). The venom of the green mamba was found to contain the most specific antagonists known for M1 and M4 receptors (m1-toxin and m4-toxin). The m2-toxin has recently been isolated which binds with high selectivity to M2 receptors (48). A number of mamba venoms have also curaremimetic α-neurotoxins (homologous to the well-known α-bungarotoxin) that bind tightly to nicotinic acetylcholine receptors (47). Nicotinic acetylcholine receptors appear to be important for a number of neurophysiological processes including cognition, learning and memory. α-conotoxins are family of Cys-enriched peptides found in several marine snails (49). They behave pharmacologically as competitive antagonists of several

nicotinic acetylcholine receptors. The anatoxin-A, an algae toxin, has also been reported to interact in acute subtoxic and naturally occurring concentrations with human α4ß2-nicotinic acetylcholine receptors (50). These are only some examples that have been found to be informative molecular probes. It is expected that many more such toxins, with unexpected specifications, will be described in the coming years, since many scorpion and other venoms remain investigated.

6. Concluding remarks

It is understood that the pathogenetic bacterium *H. pylori* is the major risk factor for gastroduodenal diseases including cancer. Infection has also been implicated in the aetiology of ischemic heart disease and hypertension, as well as chronic urticaria, rosacea, autoimmune thrombocytic purpura and diminished growth in children (51). How chronic exposure of VacA and other virulent factors leads to such health problem is a promising field of investigation.

Cytotoxin-associated gene A (CagA) is another virulent factor, which is translocated from the bacteria into gastric epithelial cells. A recent study indicates that CagA can perturb mammalian signal transduction machineries and modify cellular functions by physically interacting with a host cell protein SHP-2, SRC homology 2 domain-containing tyrosine phosphatase (52). Dysregulation of SHP-2 by CagA may induce abnormal proliferation and movement of gastric epithelial cells, promoting the acquisition of a transformed phentype (52).

Genetically well defined and manipulatable animal models are needed to dissect the molecular mechanisms that underlie a postulated sequence of *H. pylori*-associated changes in infected hosts (3,53). Transgenic mice that lack parietal cells by the use of A-fragment of diphtheria toxin has been successfully used to examine the outcome of the colonization, since a determinant of the outcome is the ability of the bacterium to attach to the gastric epithelium by expressing a variety of adhesins that recognize a number of epithelial receptors (3,54). Recently the mutation was introduced in the RPTPß gene, which abolished expression of all the three isoforms of RPTPß (55). The RPTPß-deficient mice appeared to be normal in their gross general behavior and with respect to fertility, body weight, and life span. Such mutant mice should help to determine not only how the cytotoxins are important for *H. pylori* pathogenesis in the stomach and potentially in other sites, but also how tyrosine phosphatases are important for the control of gastrointestinal functions.

References

1. Blaser MJ: *Helicobacter pylori*: microbiology of a 'slow' bacterial infection. Trends Microbiol 1: 255-260, 1993.
2. Falk PG, Syder AJ, Guruge JL, Kirschner D, Blaser MJ and Gordon JI: Theoretical and experimental approaches for studying factors defining the *Helicobacter pylori*-host relationship. Trends Microbiol 8: 321-329, 2000.
3. Cover TL: The vacuolating cytotoxin of *Helicobacter pylori*. Mol Microbiol 20: 241-246, 1996.
4. Montecucco C, Papini E, De Bernard M and Zoratti M: Molecular and cellular activities of *Helicobacter pylori* pathogenic factors. FEBS Lett 452: 16-21, 1999.
5. Marchetti M, Arico B, Burroni D, Figura N, Rappuoli R and Ghiara P: Development of a mouse model of *Helicobacter pylori* infection that mimics human disease. Science 267: 1655-1658, 1995.
6. Ye D, Willhite DC and Blanke SR: Identification of the minimal intracellular vacuolating domain of the *Helicobacter pylori* vacuolating toxin. J Biol Chem 274: 9277-9282, 1999.
7. Pagliaccia C, De Bernard M, Lupetti P, Ji X, Burroni D, Cover TL, Papini E, Rappuoli R, Telford JL and Reyrat JM: The m2 form of the *Helicobacter pylori* cytotoxin has cell type-specific vacuolating activity. Proc Natl Acad Sci USA 95: 10212-10217, 1998.
8. Seto K, Hayashi-Kuwabara Y, Yoneta T, Suda H and Tamaki H: Vacuolation induced by cytotoxin from *Helicobacter pylori* is mediated by the EGF receptor in HeLa cells. FEBS Lett 431: 347-350, 1998.
9. Cover TL: An intracellular target for *Helicobacter pylori* vacuolating toxin. Trends Microbiol 6: 127-129, 1998.
10. Papini E, Satin B, Bucci C, De Bernard M, Telford JL, Manetti R, Rappuoli R, Zerial M and Montecucco C: The small GTP binding protein rab7 is essential for cellular vacuolation induced by *Helicobacter pylori* cytotoxin. EMBO J 16: 15-24, 1997.
11. Schmid SL, McNiven MA and De Camilli P: Dynamin and its partners: a progress report. Curr Opin Cell Biol 10: 504-512, 1998.
12. Jones SM, Howell KE, Henley JR, Cao H and McNiven MA: Role of dynamin in the formation of transport vesicles from the Trans-Golgi Network. Science 279: 573-577, 1998.
13. Kreitzer G, Marmorstein A, Okamoto P, Vallee R and Rodriguez-Boulan E: Kinesin and dynamin are required for post-Golgi transport of a plasma-membrane protein. Nat Cell Biol 2: 125-127, 2000.
14. Suzuki J, Ohnishi H, Shibata H, Wada A, Hirayama T, Iiri T, Ueda N, Kanamaru C, Tsuchida T, Mashima H, Yasuda H and Fujita T: Dynamin is involved in human epithelial cell vacuolation caused by the *Helicobacter pylori*-produced cytotoxin VacA. J Clin Invest 107: 363-370, 2001.
15. De Bernard M, Moschioni M, Napolitani G, Rappuoli R and Montecucco C: The VacA toxin of *Helicobacter pylori* identifies a new intermediate filament-interacting protein. EMBO J 19: 48-56, 2000.
16. Satin B, Norais N, Telford J, Rappuoli R, Murgia M, Montecucco C and Papini E: Effect of *Helicobacter pylori* vacuolating toxin on maturation and extracellular release of procathepsin D and on epidermal growth factor degradation. J Biol Chem 272: 25022-25028, 1997.
17. Galmiche A, Rassow J, Doye A, Cagnol S, Chambard J-C, Contamin S, De Thillot V, Just I, Ricci V, Solcia E, van Obberghen E and Boquet P: The N-terminal 34 kDa fragment of *Helicobacter pylori* vacuolating cytotoxin targets mitochondria and induces cytochrome c release. EMBO J 19: 6361-6370, 2000.
18. Falnes PO and Sandvig K: Penetration of protein toxins into cells. Curr Opin Cell Biol 12: 407-413, 2000.
19. Reyrat JM, Pelicic V, Papini E, Montecucco C, Rappuoli R and Telford JL: Towards deciphering the *Helicobacter pylori* cytotoxin. Mol Microbiol 34: 197-204, 1999.
20. He D, Hagen SJ, Pothoulakis C, Chen M, Medina ND, Warny M and La Mont JT: Clostridium difficile toxin A causes early damage to mitochondria in cultured cells. Gastroenterology 119: 139-150, 2000.
21. McGivney A and Bradley SG: Action of bacterial lipopolysaccharide on the respiration of mouse liver mitochondria. Infect Immun 27: 102-106, 1980.
22. Agata N, Mori M, Ohta M, Suwan S, Ohtani I and Isobe M: A novel dodecadepsipeptide, cereulide, isolated from Bacillus cereus causes vacuole formation in HEp-2 cells. FEMS Microbiol Lett 121: 31-34, 1994.
23. Yahiro K, Niidome T, Kimura M, Hatakeyama T, Aoyagi H, Kurazono H, Imagawa K, Wada A, Moss J and Hirayama T: Activation of *Helicobacter pylori* VacA toxin by alkaline or acid conditions increases its binding to a 250-kDa receptor protein-tyrosine phosphatase ß. J Biol Chem 274: 36693-36699, 1999.
24. Padilla PI, Wada A, Yahiro K, Kimura M, Niidome T, Aoyagi H, Kumatori A, Anami M, Hayashi T, Fujisawa J, Saito H, Moss J and Hirayama T: Morphologic differentiation of HL-60 cells is associated with appearance of RPTPß and induction of *Helicobacter pylori* VacA sensitivity. J Biol Chem 275: 15200-15206, 2000.
25. Peles E, Schlessinger J and Grumet M: Multi-ligand interactions with receptor-like protein tyrosine phosphatase ß: implications for intercellular signaling. Trends Biochem Sci 23: 121-124, 1998.

26. Holland SJ, Peles E, Pawson T and Schlessinger J: Cell-contact-dependent signalling in axon growth and guidance: Eph receptor tyrosine kinases and receptor protein tyrosine phosphatase ß. Curr Opin Neurobiol 8: 117-127, 1998.
27. Meng K, Rodriguez-Pena A, Dimitrov T, Chen W, Yamin M, Noda M and Deuel TF: Pleiotrophin signals increased tyrosine phosphorylation of ß-catenin through inactivation of the intrinsic catalytic activity of the receptor-type protein tyrosine phosphatase ß/ζ. Proc Natl Acad Sci USA 97: 2603-2608, 2000.
28. Maeda N and Noda M: 6B4 proteoglycan/phosphacan is a repulsive substratum but promotes morphological differentiation of cortical neurons. Development 122: 647-658, 1996.
29. Goldin AL: Voltage gated sodium channels. In: Handbook of Receptors and Channels Series, Ligand-and Voltage-Gated Channels. Noth RA (ed). CRC, Boca Raton, FL, pp73-111, 1995.
30. Gordon D: Sodium channels as targets for neurotoxins: mode of action and interaction of neurotoxins with receptor sites on sodium channels. In: Toxins and Signal Transduction. Lazarowici P and Gutman Y (eds). Harwood, Amsterdam, pp119-149, 1997.
31. Gordon D, Savarin P, Gurevitz M and Zinn-Justin S: Functional anatomy of scorpion toxins affecting sodium channels. J Toxicol Toxin Rev 17: 131-158, 1998.
32. Shon KJ, Olivera BM, Watkins M, Jacobsen RB, Gray WR, Floresca CZ, Cruz LJ, Hillyard DR, Brink A, Terlau H and Yoshikami D: µ-Conotoxin PIIIA, a new peptide for discriminating among Tetrodotoxin-sensitive Na channel subtypes. J Neurosci 18: 4473-4481, 1998.
33. Felts PA, Yokoyama S, Dib-Hajj S, Black JA and Waxman SG: Sodium channel α-subunit mRNAs I, II, III, NaG, Na6 and hNE (PN1): different expression patterns in developing rat nervous system. Brain Res Mol Brain Res 45: 71-82, 1997.
34. Gilles N, Blanchet C, Shichor I, Zaninetti M, Lotan I, Bertrand D and Gordon D: A scorpion α-like toxin that is active on insects and mammals reveals an unexpected specificity and distribution of sodium channel subtypes in rat brain neurons. J Neurosci 19: 8730-8739, 1999.
35. Possani LD, Becerril B, Delepierre M and Tytgat J: Scorpion toxins specific for Na^{++} channels. Eur J Biochem 264: 287-300, 1999.
36. Ratcliffe CF, Qu Y, McCormick KA, Tibbs VC, Dixon JE, Scheuer T and Catterall WA: A sodium channel signaling complex: modulation by associated receptor protein tyrosine phosphatase beta. Nat Neurosci 3: 437-444, 2000.
37. Dunlap K, Luebke JI and Turner TJ: Exocytotic Ca^{2+} channels in mammalian central neurons. Trends Neurosci 18: 89-98, 1995.
38. Poncer J-C, McKinney RA, Gahwiler BH and Thompson SM: Either N- or P-type calcium channels mediate GABA release at distinct hippocampal inhibitory synapses. Neuron 18: 463-472, 1997.
39. Tsien RW, Ellinor PT and Horne WA: Molecular diversity of voltage-dependent Ca^{2+} channels. Trends Pharmacol Sci 12: 349-354, 1991.
40. Birnbaumer L, Campbell KP, Catterall WA, Harpold MM, Hofmann F, Horne WA, Mori Y, Schwartz A, Snutch TP, Tanabe T and Tsien RW: The naming of voltage-gated calcium channels. Neuron 13: 505-506, 1994.
41. Yan L and Adams ME: The spider toxin omega-Aga IIIA defines a high affinity site on neuronal high voltage-activated calcium channels. J Biol Chem 275: 21309-21316, 2000.
42. Kaczorowski GJ and Garcia ML: Pharmacology of voltage-gated and calcium-activated potassium channels. Curr Opin Chem Biol 3: 448-458, 1999.
43. Miller C: The charybdotoxin family of K channel-blocking peptides. Neuron 15: 5-10, 1995.
44. Garateix A, Vega R, Salceda E, Cebada J, Aneiros A and Soto E: BgK anemone toxin inhibits outward K^+ currents in snail neurons. Brain Res 864: 312-314, 2000.
45. Kushmerick C, Kalapothakis E, Beirao PS, Penaforte CL, Prado VF, Cruz JS, Diniz CR, Cordeiro MN, Gomez MV, Romano-Silva MA and Prado MA: Phoneutria nigriventer toxin Tx3-1 blocks A-type K^+ currents controlling Ca^{2+} oscillation frequency in GH3 cells. J Neurochem 72: 1472-1481, 1999.
46. Jerusalinsky D, Kornisiuk E, Alfaro P, Quillfeldt J, Ferreira A, Rial VE, Duran R and Cervenansky C: Muscarinic toxins: novel pharmacological tools for the muscarinic cholinergic system. Toxicon 38: 747-761, 2000.
47. Jerusalinsky D and Harvey AL: Toxins from mamba venoms: small proteins with selectivities for different subtypes of muscarinic acetylcholine receptors. Trends Pharmacol Sci 15: 424-430, 1994.
48. Carsi JM, Valentine HH and Potter LT: m2-toxin: a selective ligand for M2 muscarinic receptors. Mol Pharmacol 56: 933-937, 1999.
49. Arias HR and Blanton MP: α-conotoxins. Int J Biochem Cell Biol 32: 1017-1028, 2000.
50. Durany N, Riederer P and Deckert J: The CNS toxin anatoxin-A interacts with α4ß2-nicotinic acetylcholine receptors in human cortex. Alzheimers' Rep 2: 263-266, 1999.
51. Nabwera HM and Logan RP: Epidemiology of *Helicobacter pylori*: transmission, translocation and extragastric reservoirs. J Physiol Pharmacol 50: 711-722, 1999.
52. Higashi H, Tsutsumi R, Muto S, Sugiyama T, Azuma T, Asaka M and Hatakeyama M: SHP-2 tyrosine phosphatase as an intracellular target of *Helicobacter pylori* CagA protein. Science 295: 683-686, 2002.
53. Inui A: Transgenic study of energy homeostasis equation: implications and confounding influences. FASEB J 14: 2158-2170, 2000.
54. Syder AJ, Guruge JL, Li Q, Hu Y, Oleksiewicz CM, Lorenz RG, Karam SM, Falk PG and Gordon JI: *Helicobacter pylori* attaches to NeuAcα 2,3Galß 1,4 glycoconjugates produced in the stomach of transgenic mice lacking parietal cells. Mol Cell 3: 263-274, 1999.
55. Harroch S, Palmeri M, Rosenbluth J, Custer A, Okigaki M, Shrager P, Blum M, Buxbaum JD and Schlessinger J: No obvious abnormality in mice deficient in receptor protein tyrosine phosphatase ß. Mol Cell Biol 20: 7706-7715, 2000.