Peripheral benzodiazepine receptor and its clinical targeting

Didier Decaudin[a]

Tumor cell targeted therapies, by induction or enhancement of apoptosis, constitute recent promising approaches achieving more specific anti-tumor efficacy. The peripheral benzodiazepine receptor (PBR), which belongs to the permeability transition pore (PTP), the central regulatory complex of apoptosis, is a potential target. A number of findings argue in favor of the development of PBR targeting approaches: (i) overexpression of PBR has been described in a large range of human cancers, (ii) PTP-mediated regulation of programmed cell death is an apoptotic-inducing factor-independent check-point that could be modulated by various conventional cancer therapies, and (iii) PBR ligation enhances apoptosis induction in many types of tumors and reverses Bcl-2 cytoprotective effects. Altogether, these observations support the use of PBR-directed drugs, particularly PBR ligands such as Ro5-4864, in the treatment of human cancers. *Anti-Cancer Drugs* 15:737–745 © 2004 Lippincott Williams & Wilkins.

Anti-Cancer Drugs 2004, **15:**737–745

Keywords: apoptosis, cancer, mitochondria, ligand, peripheral benzodiazepine receptor

[a]Department of Clinical Hematology, Institut Curie, Paris, France.

Correspondence to D. Decaudin, Service d'Hématologie, Institut Curie, 26 rue d'Ulm, 75.248 Paris Cedex 05, France.
Tel: +33 1 44 32 46 90; fax: +33 1 44 32 46 93;
e-mail: didier.decaudin@curie.net

Received 4 May 2004 **Revised form accepted** 3 June 2004

Introduction

The benzodiazepine family constitutes a group of anxiolytic and anti-convulsant drugs initially described as ligands of central nervous system sites closely linked to GABA receptors and which modulate the GABA-regulated anion channel [1]. In 1977, a second type of benzodiazepine receptor was identified, the so-called peripheral benzodiazepine receptor (PBR) [2]. Two classes of benzodiazepine are therefore distinguished: one with high affinity for the central-type benzodiazepine receptor and a weak interaction with the PBR, and the other which binds to the peripheral-type benzodiazepine receptor with a nanomolar affinity and no anxiolytic or anti-convulsant activity. The mitochondrial localization of the PBR explains recent research performed on the potential anti-tumor effect of PBR ligands, as the PBR, located at the outer membrane, belongs to a multiprotein complex called the permeability transition pore (PTP) that plays a key role in control of the apoptotic process. Numerous reports have shown a direct relationship between PBR targeting and apoptosis induction, and particularly a role of PBR in regulation of programmed cell death. This review focuses on the relationship between the PBR and malignant diseases and PBR tumor cell targeting therapy approaches.

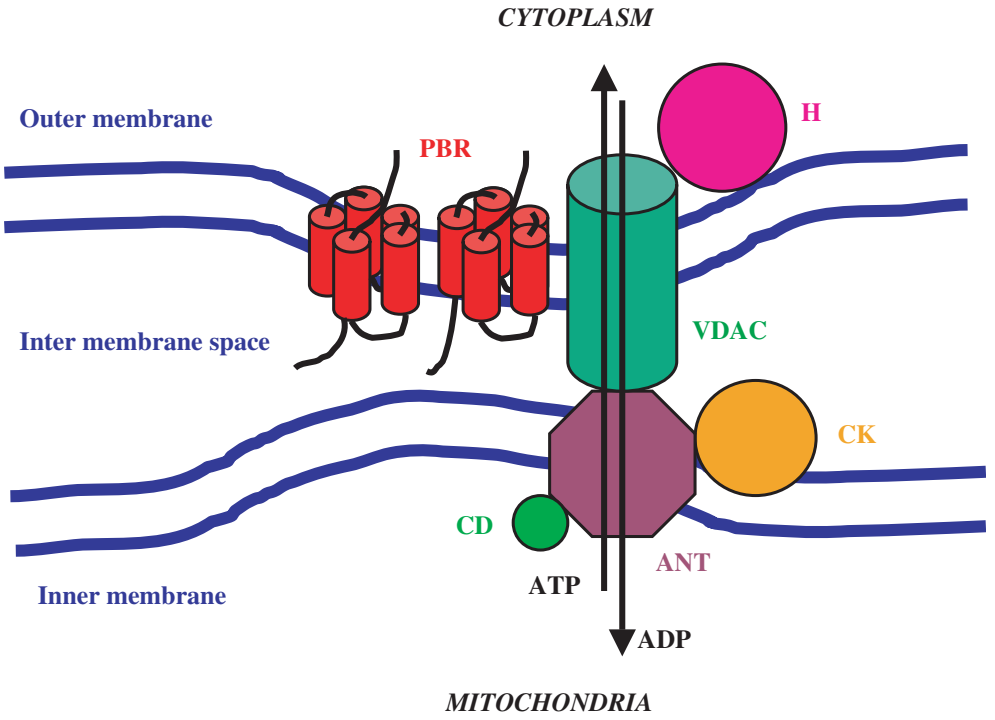
PBR structure, intracellular localization and protein interaction

PBR is an evolutionarily conserved, highly hydrophobic 18-kDa protein, for which three-dimensional modeling has shown five α helix transmembrane domains [3]. This channel-like structure is mainly located and spanned on the outer mitochondrial membrane, but PBR localization has also been detected on plasma membranes [4,5], the Golgi apparatus, lysosomes, rough endoplasmic reticular microsomes, peroxisomes [5] and nuclear membranes [6]. In mitochondria, PBR belongs to the PTP, which is a multiprotein complex located at the contact site between the mitochondrial inner and outer membranes (Fig. 1). Several proteins contribute to PTP formation, including mitochondrial hexokinase, the PBR and a porin called the voltage-dependent anion channel (VDAC) in the outer membrane, creatine kinase in the intermembrane space, the adenine nucleotide translocator (ANT) in the inner membrane, and cyclophilin D in the matrix [7,8]. Mitochondrial fraction analyses have shown that ANT and VDAC proteins are strongly associated with the PBR. A few other proteins have been found to be associated with the PBR, i.e. PRAX-1 (PBR associated protein 1) which interacts with the C-terminal end of PBR and induces its dimerization [9], a 10-kDa protein whose function remains unknown [10], two steroidogenic regulatory proteins, StAR which binds cholesterol and promotes its mitochondrial transfer [11,12] and PAP7 [13], and finally the myxoma poxvirus virulence factor M11L, the interaction with which prevents apoptosis induction via inhibition of the transmembrane mitochondrial potential drop and cytochrome *c* mitochondrial release [14]. All these PBR-associated proteins indicate the two main functions of the PBR that have now been clearly identified, i.e. regulation of steroidogenesis and the apoptotic process (Fig. 2).

Benzodiazepine receptors can be divided into two categories, i.e. central and PBRs, whose main characteristics are summarized in Table 1. Numerous PBR ligands

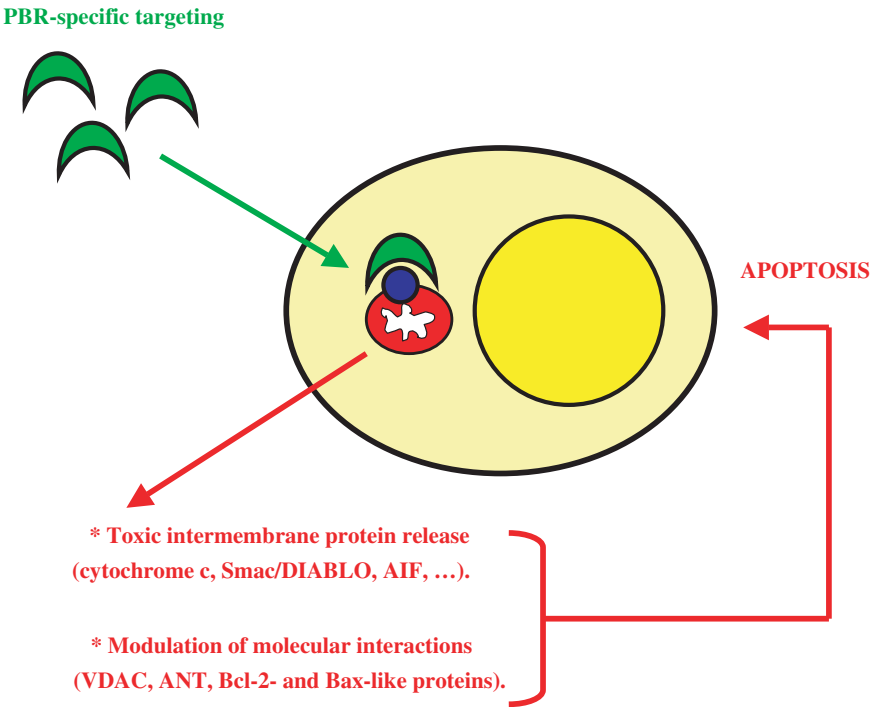
0959-4973

Fig. 1



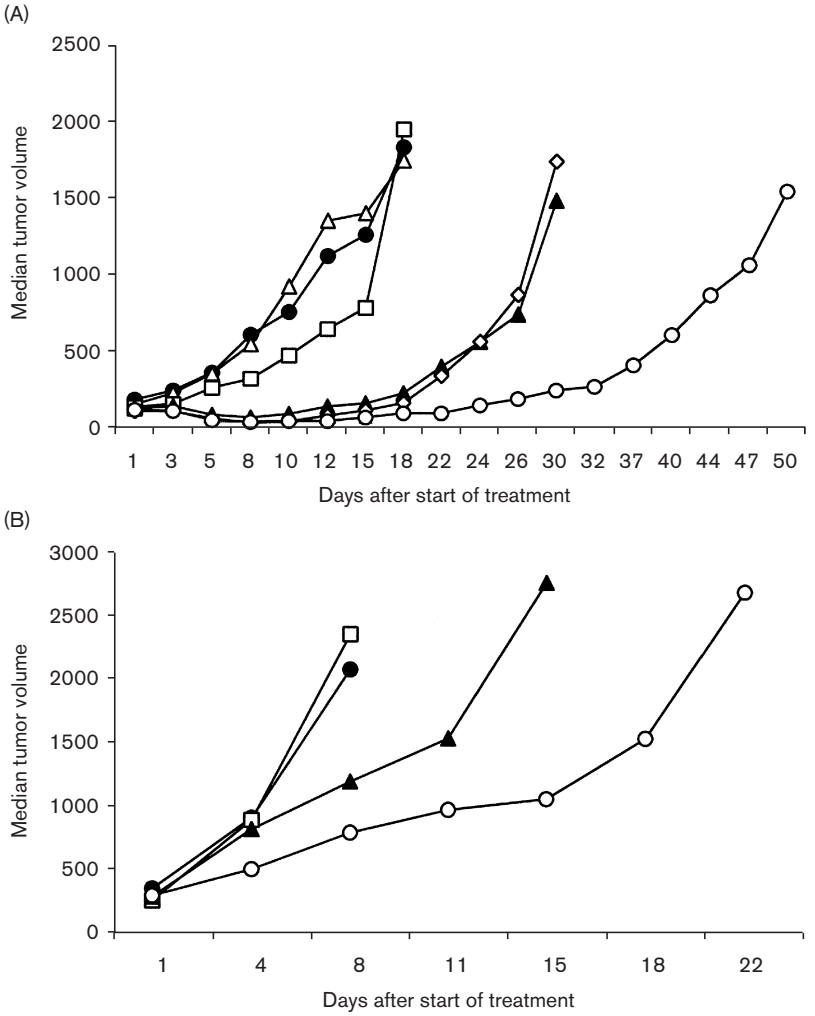
The permeability transition pore structure. Several proteins contribute to PTP formation, including mitochondrial hexokinase (H), the PBR and the VDAC in the outer membrane, creatine kinase (CK) in the intermembrane space, ANT in the inner membrane, and cyclophilin D (CD) in the matrix.

Fig. 3



PBR-specific targeting. PBR ligation induces intracellular events such as toxic mitochondrial intermembrane protein release and/or modulation of protein interactions that leads to irreversible induction of the apoptotic process.

Fig. 2



In vivo enhancement of chemotherapy-induced apoptosis by Ro5-4864 (Decaudin). (A) Xenografted SCLC61 tumors were treated by etoposide with (open circles) or without (solid triangles) Ro5-4864. Two control groups received Ro-excipient alone (open triangles) or Ro-excipient and etoposide (open diamonds). (B) Etoposide and ifosfamide were administered to *nude* mice bearing SCLC6 tumors, with (open circles) or without (solid triangles) Ro5-4864. Two control groups received injections of either Ro5-4864 (open squares) or 0.9% NaCl alone (solid circles). Tumor growth was evaluated by measuring the relative tumor volume (RTV). Statistical analyses were performed using the Student's *t*-test.

Table 1 Characteristics of central- and peripheral-type benzodiazepine receptors

	Central-type receptors	Peripheral-type receptors
Intracellular localization	plasma membrane	outer mitochondrial membrane, (plasma membrane)
Tissue distribution	Neuronal	ubiquitous
Molecular components	heterogeneous (α and β subunits of the GABA receptor)	pK18, VDAC, ANT, pK10
Physiological function	inhibitory neurotransmitter	steroidogenesis, regulation of apoptosis and inflammation processes
Endogenous ligands	DBI	DBI, porphyrins, cholesterol
Exogenous ligands	benzodiazepines (clonazepam, diazepam)	benzodiazepines (diazepam, Ro5-4864), PK11195, FGIN-1-27, SSR180575

GABA, γ-aminobutyric acid; VDAC, voltage-dependent anion channel; ANT, adenine nucleotide translocator; DBI, diazepam binding inhibitor.

have been identified: (i) endogenous ligands such as DBI ('diazepam binding inhibitor'; also called endozepine), and its derived fragments, porphyrins (protoporphyrins IX, heme) and cholesterol, and (2) synthetic ligands such as benzodiazepines (diazepam, 4′-chlorodiazepam or Ro5-4864), isoquinoline carboxamides (PK11195),

indolacetamide derivatives (FGIN-1-27) and pyrisanoindole derivatives (SSR180575).

PBR expression in normal and tumor tissues

PBR is ubiquitously expressed in normal tissues and organs, but with various receptor densities. In particular, a high level of PBR expression has been observed in the convoluted tubules and ascending loop of Henle of the kidney, the adrenal cortex, and afferent olfactory nerves in the brain. This expression was correlated with high concentrations of mitochondria in the same areas [1]. In contrast, low PBR expression is observed in the liver and other parts of the brain. This modulated expression could be affected by numerous physiological or physiological-like factors, such as hormonal regulation (the gonadotrophin-releasing hormone agonist decapeptyl inducing pharmacological castration), catecholamines, interleukin (IL)-1β, tumor necrosis factor (TNF)-α and interferon (IFN)-γ [15–20]. Similarly, PBR levels are significantly increased by non-neoplastic diseases, such as brain [21], kidney [22] and myocardial [23] ischemia, brain damage induced by toxins [24,25], viral encephalitis [26], hepatic encephalopathy [27], epilepsy [28], nerve degeneration [29], and trauma [30]. Moreover, lithium and antidepressants induce decreased PBR expression, while diuretics upregulate PBR levels [31,32]. Finally, neutrophil PBR expression is altered in X-linked chronic granulomatous disease [33] and this expression can be restored by IFN-γ [34].

Considerable data are available concerning PBR tumor cell line expression, as PBR overexpression has been described in various breast [35–40], Leydig [41,42], esophageal [43], colorectal [44], ovarian [45], osteosarcoma [46], neuroblastoma [46], T cell lymphoid [46], prostatic [47], small cell lung cancer [46] and glioma [46,48] cancer cell lines. In contrast to the published data on tumor cell lines, very few data have been reported concerning PBR expression of human cancers *in vivo*. PBR overexpression has been reported in hepatocellular carcinoma [49], ovarian cancer [50], astrocytomas [51], endometrial carcinoma [52] and colorectal cancers [53]. Moreover, a pejorative prognostic impact of PBR overexpression has been found in stage III colorectal tumors [53] and breast cancer cell lines [6]. However, it has not been determined whether PBR upregulation is a characteristic of numerous cancers, and whether PBR overexpression constitutes an independent prognostic factor for chemotherapy-induced responses.

Functions of PBR

Two main functions of PBR have been described: a role in steroidogenesis and modulation of the apoptotic process, with few recent implications in other physiological or pathological domains. The next part of this review will focus on PBR regulation of apoptosis.

The PBR function was first identified in steroidogenesis. In this situation, PBR binds cholesterol and mediates its transport from the outer to the inner mitochondrial membranes [3,41,54–58]. This translocation results in increasing pregnenolone formation and finally steroidogenesis. Molecular modeling of PBR has suggested that cholesterol might cross the membrane via the five α helix of the receptor, and that synthetic and endogenous ligands might bind to common sites in the cytoplasmic loops. *In vitro* reconstitution of PBR in proteoliposomes demonstrated that PBR binds both drug ligands and cholesterol with high affinity [59]. It has been shown that the PBR polymer might be the functional unit responsible for ligand-activated cholesterol binding, and that PBR polymerization is a dynamic process modulating the function of this receptor in cholesterol transport and other cell-specific PBR-mediated functions [60]. These data indicate that hormone-induced cholesterol transport and subsequent steroid formation is a dynamic multistep process involving protein–protein interactions [61]. This PBR function is particularly crucial in brain neurosteroid biosynthesis [62–64]. In this context, a correlation has been observed between PBR expression, steroid biosynthesis and oligodendrocyte differentiation, suggesting a role of the PBR in neurologic development and differentiation processes [65]. Concurrently, PBR overexpression described in numerous neuropathologies, such as Alzheimer's disease [66] and Huntington's disease [67], is associated with an increase of pregnenolone levels in pathological brain regions [28,68,69]. Various reports have therefore suggested that stimulation of neurosteroid synthesis, combined with promotion of neuronal cell survival via regulation of apoptosis, participate to support the survival of nerves affected by neurodegenerative diseases.

PBR is involved in human cancer cell proliferation, as a relationship between cell proliferation and PBR expression has been observed in human astrocytomas [51] and breast cancer cell lines [36,37]. Similarly, it has been reported that PBR ligands induce *in vitro* inhibition of cancer cell proliferation [35,43,44,46,70–74], and that *in vivo* formation of human breast tumor in SCID mice may depend on the amount of PBR present in the cells [75]. This antiproliferative effect is mediated by mitosis arrest in the G_2/M stage without affecting DNA synthesis [72]. Another mechanism to explain PBR-induced modulation of cell proliferation is supported by the various subcellular localization of the PBR, as PBR detected in and around the nucleus of human glioma and breast cancer cell lines was associated with aggressive tumors and high proliferative index [6,76]. Moreover, PBR-dependent cell proliferation was found to be strongly correlated with PBR-mediated changes in nuclear membrane cholesterol levels. Altogether, these results indicate that the nuclear localization of PBR increases cholesterol transport into the nucleus, and intranuclear cholesterol levels have been

shown to increase cell proliferation and cancer progression [77].

Some other publications have provided further evidence for a role played by PBR in regulation of inflammation processes, as various *in vivo* mouse models of acute inflammation have shown that PBR ligands inhibit inflammatory signs of pleurisy [78], arthritis [79] or lupus erythematosus [80]. In this context, various observations have been reported to explain the mechanisms by which PBR modulates inflammation responses, i.e. (i) modulation of the human natural killer cell activity [81], (ii) induction of heat shock protein expression [82], (iii) modulation of the activity of monocytes/macrophages [83–85] and (iv) restoration of the apoptotic process in auto-immune components [86]. Finally, several other functions of PBR have been identified, i.e. regulation of ischemia-reperfusion injury via membrane biogenesis [64,87], protection of hematopoietic cells against oxygen radical damage [88], lipid fluidity of mitochondria [89] and modulation of bronchomotor tone [90]. PBR has also been reported to play a role in erythroid differentiation [91], and a role in the intracellular transport of heme and porphyrins, similar to the effect observed in steroidogenesis [92,93].

PBR and regulation of apoptosis

The apoptotic process is marked by a series of morphological and molecular alterations, including disruption of mitochondrial membrane integrity, caspase activation and DNA fragmentation [8]. Over recent years, it has been widely accepted that apoptosis is under the control of mitochondria and that the PTP plays a key role in this regulation [94]. Mitochondrial membrane permeabilization (MMP) therefore appears to be a major checkpoint in the cascade of biochemical events leading to the induction of programmed cell death, as it has been demonstrated that a number of apoptosis-inducing signals induce MMP and anti-apoptotic proteins also block this alteration [95]. The loss of mitochondrial membrane integrity leads to a drop of transmembrane potential and remodeling of mitochondrial ultrastructure that allow the release of toxic intermembrane proteins into the cytoplasm such as cytochrome *c*, Smac/DIABLO, AIF (apoptosis-inducing factor) and endonuclease-G [96]. These apoptotic effectors are then responsible for the late events of the cell death process. In this cascade of molecular events, MMP appears to be an irreversible step which commits the cell to undergo death, suggesting that MMP marks the point-of-no-return of apoptosis [95].

Numerous observations indicate that PBR participates in the regulation of apoptosis: (i) transfection-enforced overexpression of PBR attenuates apoptosis induced by oxygen radicals or ultraviolet light [97,98], (ii) permeabilized mitochondria release DBI that binds intact mitochondria and accelerates MMP induction throughout the cell [99], (iii) the myxoma poxvirus M11L protein inhibits host cell apoptosis via a physical and functional interaction with PBR [100], and (iv) various PBR ligands with nanomolar affinity for the receptor, such as Ro5-4864 and PK11195, modulate cancer cell response to apoptosis-inducing signals (Table 2) [39,44,46,101–110]. This effect has been observed in several histologic types

Table 2 Effect of PBR ligands on apoptosis induction

Reference	Type of tumor	Type of PBR ligand	*In vitro* study	*In vivo* study	Effect on apoptosis	With other cancer treatments
101	glioblastoma cells	PBR–MEL conjugate	+	0	+	melphalan
102	thymocyte, B and T lymphoid cells	PK11195	+	0	+	glucocorticoid; etoposide; doxorubicin; γ-irradiation; ceramide
103	glioblastoma	diazepam	+	+	+	lonidamide
104	lymphoblastoid cells	Ro5-4864	+	0	–	TNF-α
105	murine thymocytes	peripheral-type benzodiazepines	+	0	+	dexamethasone; etoposide
106	murine leukemia cells	PP-IX	+	0	+	photodynamic therapy
44	colorectal cancer	FGIN-1-27; PK11195; Ro5-4864	+	0	+	0
107	acute myeloid leukemia	PK11195	+	0	+	daunomycin; cytarabine
46	T cells, neuroblastoma, osteosarcoma, glioblastoma, small cell lung cancer	Ro5-4864; PK11195; diazepam	+	+	+	anti-CD95 monoclonal antibody; etoposide; ifosfamide
108	myeloid leukemia and ovarian carcinoma	PK 11195	+	0	+	daunomycin
109	Chinese hamster ovary cells	Pc4	+	0	+	photodynamic therapy
39	breast cancer	Ro5-4684; PK11195	+	0	+	tamoxifen
110	esophageal cancer	FGIN-1-27	+	0	+	0

PP-IX, protoporphyrin-IX; Pc4, the phthalocyanine photosensitizer which accumulates in mitochondria and structurally resembles porphyrins.

of neoplasms, suggesting that it is not dependent on tumor-cell specificity. It has also been demonstrated that enhancement of apoptosis induction by PBR ligands requires concomitant exposure to PBR ligands and apoptosis-inducing factor [46], and that Bcl-2-induced resistance can be overcome by PBR ligation in a number of *in vitro* Bcl-2-overexpressing models [46,100,102,109]. PBR ligand-induced enhancement of apoptosis clearly acts via mitochondrial targeting, as demonstrated by experiments performed on isolated organelles or cell cultures, showing cytochrome *c* and Smac/DIABLO release, and caspase 9 and 3 activation [46,102]. Finally, PBR ligands combined with cytotoxic agents have an anti-tumor effect in several *in vivo* models: Ro5-4864 increased tumor growth inhibition induced by etoposide or a combination of etoposide plus ifosfamide of human small cell lung cancer xenografted tumors into *nude* mice (Fig. 3, see p. 738) [46], and diazepam cooperated with lonidamine to inhibit the growth of human xenografted glioblastoma tumors [103].

Bono *et al.* showed that PK11195 enhanced the sensitivity of cells to TNF-α and abolished the apoptosis-inhibitory effect of Bcl-2 via a direct effect on mitochondria [104]. However, the same authors reported that, at concentrations between 10 and 100 nM, Ro5-4864 reduced the pro-apoptotic effect of TNF-α. This observation is not in agreement with data showing that, at concentrations lower than 1 μM, Ro5-4864, PK11195 and diazepam, failed to stimulate CD95-induced apoptosis [46]. This apparent discrepancy could be explained by the fact that the two PBR ligands, Ro5-4864 (a benzodiazepine) and PK11195 (an isoquinoline carboxamide), exert different conformational and/or physicochemical changes on their mitochondrial binding partners. The differential effects observed with Ro5-4864 and PK11195 support the assumption that the two ligands act as an agonist and as an antagonist of PBR, respectively [111], and that they interact with two different conformations or domains of the mitochondrial PBR [112]. It could be therefore speculated that the binding of the PBR ligand to its receptor induces a peculiar conformation of the mitochondrial permeability transition pore, which sensitizes the cell to an apoptotic message. PBR could also modulate apoptosis via direct molecular interactions with the PTP components, i.e. VDAC and ANT, and/or anti-apoptotic Bcl-2- and pro-apoptotic Bax-like proteins. The PTP therefore appears to be a multiprotein complex whose molecular dynamics could be influenced by several partners. PBR is one of these partners and could therefore be used as a target in clinical and therapeutic approaches.

Clinical PBR targeting

A number of findings argue in favor of the development of PBR targeting approaches in the treatment of human cancers. (i) PBR overexpression has been observed in a large variety of human cancers. (ii) PBR is a component of the central regulatory complex of apoptosis, i.e. the mitochondrial PTP, which acts as an irreversible checkpoint of programmed cell death induction. This regulation remains independent of apoptotic-inducing factors, suggesting that PBR targeting could be of interest in combination with various anti-tumor therapies. (iii) PBR binding by high-affinity ligands enhances apoptosis induction of numerous inducers, such as cytotoxic agents, monoclonal antibodies and ionizing radiation. This effect has been demonstrated in various types of human tumors, *in vitro* and *in vivo*. Moreover, PBR ligands are able to reverse the Bcl-2 cytoprotective effect. In the context of tumor cell targeted therapies, PBR constitutes a tumor-specific intracellular component which interferes with the regulation of programmed cell death and binding to PBR induces tumor cell apoptosis. Altogether, these observations are therefore sufficient to justify the use of PBR ligands in combination with conventional anti-tumor therapies, as already tested in small cell lung cancers. In these clinical situations, it could be of interest to evaluate, together with the level of PBR expression, prognostic factors that influence response to these types of combined therapeutic modalities of PBR ligands and conventional therapies. The determination of gene expression patterns by DNA microarray experiments could be crucial to elucidate the mechanisms that participate in the apoptosis-regulatory effect of the PBR and subsequently optimize PBR-mediated therapeutic enhancement. Lastly, PBR ligation could be associated with other specific targeted approaches that may cooperate and bypass tumor cell resistance in order to obtain more effective cancer cell eradication. However, this enthusiasm must be modulated by the need to determine whether PBR ligands are non-toxic to normal cells, and to evaluate the possibility of concomitant administration of both PBR ligands and cytotoxic treatments in cancer patients. This clinical research, based on fundamental observations concerning the basic biological mechanisms of the cell and the specificities of cancer cells, offers an attractive perspective for the management of human cancers. PBR appears to be a functional unit that should certainly be a subject of interest to the scientific community in the context of tumor-specific targeting.

References

1 Anholt RR, Pedersen PL, De Souza EB, Snyder SH. The peripheral-type benzodiazepine receptor. Localization to the mitochondrial outer membrane. *J Biol Chem* 1986; **261**:576–583.

2 Braestrup C, Squires RF. Specific benzodiazepine receptors in rat brain characterized by high-affinity [^{3}H]diazepam binding. *Proc Natl Acad Sci USA* 1977; **74**:3805–3809.

3 Bernassau JM, Reversat JL, Ferrara P, Caput D, Lefur G. A 3D model of the peripheral benzodiazepine receptor and its implication in intra mitochondrial cholesterol transport. *J Mol Graphics* 1993; **11**:236–245.

4 Olson JM, Ciliax BJ, Mancini WR, Young AB. Presence of peripheral-type benzodiazepine binding sites on human erythrocyte membranes. *Eur J Pharmacol* 1988; **152**:47–53.

5 O'Beirne GB, Woods MJ, Williams DC. Two subcellular locations for peripheral-type benzodiazepine acceptors in rat liver. *Eur J Biochem* 1990; **188**:131–138.

6 Hardwick M, Fertikh D, Culty M, Li H, Vidic B, Papadopoulos V. Peripheral-type benzodiazepine receptor (PBR) in human breast cancer: correlation of breast cancer cell aggressive phenotype with PBR expression, nuclear localization, and PBR-mediated cell proliferation and nuclear transport of cholesterol. *Cancer Res* 1999; **59**:831–842.

7 Zoratti M, Szabo I. The mitochondrial permeability transition. *Biochim Biophys Acta* 1995; **1241**:139–176.

8 Kroemer G, Zamzami N, Susin SA. Mitochondrial control of apoptosis. *Immunol Today* 1997; **18**:44–51.

9 Galiegue S, Jbilo O, Combes T, Bribes E, Carayon P, Le Fur G, *et al.* Cloning and characterization of PRAX-1. A new protein that specifically interacts with the peripheral benzodiazepine receptor. *J Biol Chem* 1999; **270**:2938–2952.

10 Blahos 2nd J, Whalin ME, Krueger KE. Identification and purification of a 10-kilodalton protein associated with mitochondrial benzodiazepine receptors. *J Biol Chem* 1995; **270**:20285–20291.

11 Li H, Yao Z, Degenhardt B, Teper G, Papadopoulos V. Cholesterol binding at the cholesterol recognition/interaction amino acid consensus (CRAC) of the peripheral-type benzodiazepine receptor and inhibition of steroidogenesis by an HIV TAT-CRAC peptide. *Proc Natl Acad Sci USA* 2001; **98**:1267–1272.

12 West LA, Horvat RD, Roess DA, Barisas BG, Juengel JL, Niswender GD. Steroidogenic acute regulatory protein and peripheral-type benzodiazepine receptor associate at the mitochondrial membrane. *Endocrinology* 2001; **142**:502–505.

13 Li H, Degenhardt B, Tobin D, Yao ZX, Tasken K, Papadopoulos V. Identification, localization, and function in steroidogenesis of PAP7: a peripheral-type benzodiazepine receptor- and PKA (RIalpha)-associated protein. *Mol Endocrinol* 2001; **15**:2211–2228.

14 Everett H, Barry M, Lee SF, Sun X, Graham K, Stone J, *et al.* M11L: a novel mitochondria-localized protein of myxoma virus that blocks apoptosis of infected leukocytes. *J Exp Med* 2000; **191**:1487–1498.

15 Moynagh PN, O'Neill LA, Williams DC. Interleukin-1 and phorbol myristate acetate modulate the peripheral-type benzodiazepine receptor in lymphocytes and glial cells. *Biochem Pharmacol* 1993; **46**:821–827.

16 Itzhak Y, Norenberg MD. Regulation of peripheral-type benzodiazepine receptors in cultured astrocytes by monoamine and amino acid neurotransmitters. *Brain Res* 1994; **660**:346–348.

17 Sridaran R, Philip GH, Li H, Culty M, Liu Z, Stocco DM, *et al.* GnRH agonist treatment decreases progesterone synthesis, luteal peripheral benzodiazepine receptor mRNA, ligand binding and steroidogenic acute regulatory protein expression during pregnancy. *J Mol Endocrinol* 1999; **22**:45–54.

18 Rey C, Mauduit C, Naureils O, Benahmed M, Louisot P, Gasnier F. Up-regulation of mitochondrial peripheral benzodiazepine receptor expression by tumor necrosis factor alpha in testicular leydig cells. Possible involvement in cell survival. *Biochem Pharmacol* 2000; **60**:1639–1646.

19 Golani I, Weizman A, Leschiner S, Spanier I, Eckstein N, Limor R, *et al.* Hormonal regulation of peripheral benzodiazepine receptor binding properties is mediated by subunit interaction. *Biochemistry* 2001; **40**:10213–10222.

20 Trincavelli ML, Marselli L, Falleni A, Gremigni V, Ragge E, Dotta F, *et al.* Upregulation of mitochondrial peripheral benzodiazepine receptor expression by cytokine-induced damage of human pancreatic islets. *J Cell Biochem* 2002; **84**:636–644.

21 Rao VL, Bowen KK, Rao AM, Dempsey RJ. Up-regulation of the peripheral-type benzodiazepine receptor expression and [^{3}H]PK11195 binding in gerbil hippocampus after transient forebrain ischemia. *J Neurosci Res* 2001; **64**:493–500.

22 Hauet T, Han Z, Wang Y, Hameury F, Jayle C, Gibelin H, *et al.* Modulation of peripheral-type benzodiazepine receptor levels in a reperfusion injury pig kidney-graft model. *Transplantation* 2002; **74**:1507–1515.

23 Mazzone A, Mazzucchelli I, Vezzoli M, Ottini E, Auguadro C, Serio A, *et al.* Increased expression of peripheral benzodiazepine receptors on leukocytes in silent myocardial ischemia. *J Am Coll Cardiol* 2000; **36**: 746–750.

24 Kuhlmann AC, Guilarte TR. Regional and temporal expression of the peripheral benzodiazepine receptor in MPTP neurotoxicity. *Toxicol Sci* 1999; **48**:107–116.

25 Hazell AS, Normandin L, Nguyen B, Kennedy G. Upregulation of 'peripheral-type' benzodiazepine receptors in the globus pallidus in a sub-acute rat model of manganese neurotoxicity. *Neurosci Lett* 2003; **349**:13–16.

26 Mankowski JL, Queen SE, Tarwater PJ, Adams RJ, Guilarte TR. Elevated peripheral benzodiazepine receptor expression in simian immunodeficiency virus encephalitis. *J Neurovirol* 2003; **9**:94–100.

27 Haussinger D, Schliess F, Kircheis G. Pathogenesis of hepatic encephalopathy. *J Gastroenterol Hepatol* 2002; **17**:S256–S259.

28 Sauvageau A, Desjardins P, Lozeva V, Rose C, Hazell AS, Bouthillier A, *et al.* Increased expression of 'peripheral-type' benzodiazepine receptors in human temporal lobe epilepsy: implications for PET imaging of hippocampal sclerosis. *Metab Brain Dis* 2002; **17**:3–11.

29 Lacor P, Gandolfo P, Tonon MC, Brault E, Dalibert I, Schumacher M, *et al.* Regulation of the expression of peripheral benzodiazepine receptors and their endogenous ligands during rat sciatic nerve degeneration and regeneration: a role for PBR in neurosteroidogenesis. *Brain Res* 1999; **815**:70–80.

30 Raghavendra Rao VL, Dogan A, Bowen KK, Dempsey RJ. Traumatic brain injury leads to increased expression of peripheral-type benzodiazepine receptors, neuronal death, and activation of astrocytes and microglia in rat thalamus. *Exp Neurol* 2000; **161**:102–114.

31 Lukeman DS, Vaughn DA, Fanestil DD. Selective pharmacological modulation of renal peripheral-type benzodiazepine binding by treatment with diuretic drugs. *Life Sci* 1988; **42**:367–373.

32 Leschiner S, Weizman R, Shoukrun R, Veenman L, Gavish M. Tissue-specific regulation of the peripheral benzodiazepine receptor by antidepressants and lithium. *Neuropsychobiology* 2000; **42**:127–134.

33 Zavala F, Veber F, Descamps-Latscha B. Altered expression of neutrophil peripheral benzodiazepine receptor in X-linked chronic granulomatous disease. *Blood* 1990; **76**:184–188.

34 Zavala F, Veber F, Taupin V, Nguyen AT, Descamps-Latscha B. Reconstitution of peripheral benzodiazepine receptor expression in X-linked chronic granulomatous disease by interferon-gamma. *Lancet* 1990; **336**:758–759.

35 Carmel I, Fares FA, Leschiner S, Scherubl H, Weisinger G, Gavish M. Peripheral-type benzodiazepine receptors in the regulation of proliferation of MCF-7 human breast carcinoma cell line. *Biochem Pharmacol* 1999; **58**:273–278.

36 Beinlich A, Strohmeier R, Kaufmann M, Kuhl H. Relation of cell proliferation to expression of peripheral benzodiazepine receptors in human breast cancer cell lines. *Biochem Pharmacol* 2000; **60**:397–402.

37 Papadopoulos V, Kapsis A, Li H, Amri H, Hardwick M, Culty M, *et al.* Drug-induced inhibition of the peripheral-type benzodiazepine receptor expression and cell proliferation in human breast cancer cells. *Anticancer Res* 2000; **20**:2835–2847.

38 Sanger N, Strohmeier R, Kaufmann M, Kuhl H. Cell cycle-related expression and ligand binding of peripheral benzodiazepine receptor in human breast cancer cell lines. *Eur J Cancer* 2000; **36**: 2157–2163.

39 Strohmeier R, Roller M, Sanger N, Knecht R, Kuhl H. Modulation of tamoxifen-induced apoptosis by peripheral benzodiazepine receptor ligands in breast cancer cells. *Biochem Pharmacol* 2002; **64**:99–107.

40 Hardwick M, Cavalli LR, Barlow KD, Haddad BR, Papadopoulos V. Peripheral-type benzodiazepine receptor (PBR) gene amplification in MDA-MB-231 aggressive breast cancer cells. *Cancer Genet Cytogenet* 2002; **139**:48–51.

41 Papadopoulos V, Amri H, Li H, Boujrad N, Vidic B, Garnier M. Targeted disruption of the peripheral-type benzodiazepine receptor gene inhibits steroidogenesis in the R2C Leydig tumor cell line. *J Biol Chem* 1997; **272**:32129–32135.

42 Rao RM, Jo Y, Babb-Tarbox M, Syapin PJ, Stocco DM. Regulation of steroid hormone biosynthesis in R2C and MA-10 Leydig tumor cells: role of the cholesterol transfer proteins StAR and PBR. *Endocr Res* 2002; **28**: 387–394.

43 Sutter AP, Maaser K, Hopfner M, Barthel B, Grabowski P, Faiss S, *et al.* Specific ligands of the peripheral benzodiazepine receptor induce apoptosis and cell cycle arrest in human esophageal cancer cells. *Int J Cancer* 2002; **102**:318–327.

44 Maaser K, Hopfner M, Jansen A, Weisinger G, Gavish M, Kozikowski AP, *et al.* Specific ligands of the peripheral benzodiazepine receptor induce apoptosis and cell cycle arrest in human colorectal cancer cells. *Br J Cancer* 2001; **85**:1771–1780.

45 Thompson WE, Branch A, Whittaker JA, Lyn D, Zilberstein M, Mayo KE, *et al.* Characterization of prohibitin in a newly established rat ovarian granulosa cell line. *Endocrinology* 2001; **142**:4076–4085.

46 Decaudin D, Castedo M, Nemati F, Beurdeley-Thomas A, De Pinieux G, Caron A, *et al.* Peripheral benzodiazepine receptor ligands reverse apoptosis resistance of cancer cells *in vitro* and *in vivo*. *Cancer Res* 2002; **62**:388–393.

47 Batra S, Alenfall J. Characterization of peripheral benzodiazepine receptors in rat prostatic adenocarcinoma. *Prostate* 1994; **24**:269–278.

48 Whittle IR, Kelly PA. Mechanisms of peritumoural brain dysfunction: metabolic and neuroreceptor findings in striatal C6 glioma. *J Clin Neurosci* 2001; **8**:430–434.

49 Venturini I, Zeneroli ML, Corsi L, Avallone R, Farina F, Alho H, *et al.* Up-regulation of peripheral benzodiazepine receptor system in hepatocellular carcinoma. *Life Sci* 1998; **63**:1269–1280.

50 Batra S, Iosif CS. Elevated concentrations of mitochondrial peripheral benzodiazepine receptors in ovarian tumors. *Int J Oncol* 1998; **12**: 1295–1298.

51 Miettinen H, Kononen J, Haapasalo H, Helen P, Sallinen P, Harjuntausta T, *et al.* Expression of peripheral-type benzodiazepine receptor and diazepam binding inhibitor in human astrocytomas: relationship to cell proliferation. *Cancer Res* 1995; **55**:2691–2695.

52 Batra S, Iosif CS. Peripheral benzodiazepine receptor in human endometrium and endometrial carcinoma. *Anticancer Res* 2000; **20**: 463–466.

53 Maaser K, Grabowski P, Sutter AP, Hopfner M, Foss HD, Stein H, *et al.* Overexpression of the peripheral benzodiazepine receptor is a relevant prognostic factor in stage III colorectal cancer. *Clin Cancer Res* 2002; **8**:3205–3209.

54 Garnier M, Boujrad N, Ogwuegbu SO, Hudson Jr JR, Papadopoulos V. The polypeptide diazepam-binding inhibitor and a higher affinity mitochondrial peripheral-type benzodiazepine receptor sustain constitutive steroidogenesis in the R2C Leydig tumor cell line. *J Biol Chem* 1994; **269**:22105–22112.

55 Tsankova V, Magistrelli A, Cantoni L, Tacconi MT. Peripheral benzodiazepine receptor ligands in rat liver mitochondria: effect on cholesterol translocation. *Eur J Pharmacol* 1997; **294**:601–607.

56 Li H, Papadopoulos V. Peripheral-type benzodiazepine receptor function in cholesterol transport. Identification of a putative cholesterol recognition/ interaction amino acid sequence and consensus pattern. *Endocrinology* 1998; **139**:4991–4997.

57 Papadopoulos V, Widmaier EP, Amri H, Zilz A, Li H, Culty M, *et al. In vivo* studies on the role of the peripheral benzodiazepine receptor (PBR) in steroidogenesis. *Endocr Res* 1998; **24**:479–487.

58 Culty M, Li H, Boujrad N, Amri H, Vidic B, Bernassau JM, *et al. In vitro* studies on the role of the peripheral-type benzodiazepine receptor in steroidogenesis. *J Steroid Biochem Mol Biol* 1999; **69**:123–130.

59 Lacapere JJ, Papadopoulos V. Peripheral-type benzodiazepine receptor: structure and function of a cholesterol-binding protein in steroid and bile acid biosynthesis. *Steroids* 2003; **68**:569–585.

60 Delavoie F, Li H, Hardwick M, Robert JC, Giatzakis C, Peranzi G, *et al. In vivo* and *in vitro* peripheral-type benzodiazepine receptor polymerization: functional significance in drug ligand and cholesterol binding. *Biochemistry* 2003; **42**:4506–4519.

61 Hauet T, Liu J, Li H, Gazouli M, Culty M, Papadopoulos V. PBR, StAR, and PKA: partners in cholesterol transport in steroidogenic cells. *Endocr Res* 2002; **28**:395–401.

62 Lacor P, Gandolfo P, Tonon MC, Brault E, Dalibert I, Schumacher M, *et al.* Regulation of the expression of peripheral benzodiazepine receptors and their endogenous ligands during rat sciatic nerve degeneration and regeneration: a role for PBR in neurosteroidogenesis. *Brain Res* 1999; **815**:70–80.

63 Lang S. The role of peripheral benzodiazepine receptors (PBRs) in CNS pathophysiology. *Curr Med Chem* 2002; **9**:1411–1415.

64 Papadopoulos V. Peripheral benzodiazepine receptor: structure and function in health and disease. *Ann Pharm Fr* 2003; **61**:30–50.

65 Cascio C, Brown RC, Liu Y, Han Z, Hales DB, Papadopoulos V. Pathways of dehydroepiandrosterone formation in developing rat brain glia. *J Steroid Biochem Mol Biol* 2000; **75**:177–186.

66 McGeer EG, Singh EA, McGeer PL. Peripheral-type benzodiazepine binding in Alzheimer disease. *Alzheim Dis AssDisord* 1988; **2**: 331–336.

67 Messmer K, Reynolds GP. Increased peripheral benzodiazepine binding sites in the brain of patients with Huntington's disease. *Neurosci Lett* 1998; **241**:53–56.

68 Cagnin A, Brooks DJ, Kennedy AM, Gunn RN, Myers R, Turkheimer FE, *et al. In-vivo* measurement of activated microglia in dementia. *Lancet* 2001; **359**:461–467.

69 Brown RC, Han Z, Cascio C, Papadopoulos V. Neurosteroids: oxidative stress mediated dehydroepiandrosterone formation in Alzheimer's disease pathology. *Neurobiol Aging* 2003; **24**:57–65.

70 Laird HE II, Gerrish KE, Duerson KC, Putnam CW, Russell DH. Peripheral benzodiazepine binding sites in Nb 2 node lymphoma cells: effects on prolactin-stimulated proliferation and ornithine decarboxylase activity. *Eur J Pharmacol* 1989; **171**:25–35.

71 Garnier M, Boujrad N, Oke BO, Brown AS, Riond J, Ferrara P, *et al.* Diazepam binding inhibitor is a paracrine/autocrine regulator of Leydig cell proliferation and steroidogenesis: action via peripheral-type benzodiazepine receptor and independent mechanisms. *Endocrinology* 1993; **132**:444–458.

72 Camins A, Diez-Fernandez C, Pujadas E, Camarasa J, Escubedo E. A new aspect of the antiproliferative action of peripheral-type benzodiazepine receptor ligands. *Eur J Pharmacol* 1995; **272**:289–292.

73 Neary JT, Jorgensen SL, Oracion A, Bruce JH, Norenberg MD. Inhibition of growth factor-induced DNA synthesis in astrocytes by ligands of peripheral-type benzodiazepine receptors. *Brain Res* 1995; **675**:27–30.

74 Landau M, Weizman A, Zoref-Shani E, Beery E, Wasseman L, Landau O, *et al.* Antiproliferative and differentiating effects of benzodiazepine receptor ligands on B16 melanoma cells. *Biochem Pharmacol* 1998; **56**:1029–1034.

75 Hardwick M, Rone J, Han Z, Haddad B, Papadopoulos V. Peripheral-type benzodiazepine receptor levels correlate with the ability of human breast cancer MDA-MB-231 cell line to grow in SCID mice. *Int J Cancer* 2001; **94**:322–327.

76 Brown RC, Degenhardt B, Kotoula M, Papadopoulous V. Location-dependent role of the human glioma cell peripheral-type benzodiazepine receptor in proliferation and steroid biosynthesis. *Cancer Lett* 2000; **156**:125–132.

77 Coleman PS, Chen LC, Sepp-Lorenzino L. Cholesterol metabolism and tumor cell proliferation. In: Bittman R (editor): *Cholesterol: Its Functions and Metabolism in Biology and Medicine*. New York: Plenum; 1997, pp. 363–435.

78 Torres SR, Frode TS, Nardi GM, Vita N, Reeb R, Ferrara P, *et al.* Anti-inflammatory effects of peripheral benzodiazepine receptor ligands in two mouse models of inflammation. *Eur J Pharmacol* 2000; **408**:199–211.

79 Waterfield JD, McGeer EG, McGeer PL. The peripheral benzodiazepine receptor ligand PK11195 inhibits arthritis in the MRL-*lpr* mouse model. *Rheumatology* 1999; **38**:1068–1073.

80 Bribes E, Bourrie B, Esclangon M, Galiegue S, Vidal H, Casellas P. Involvement of the peripheral benzodiazepine receptor in the development of rheumatoid arthritis in Mrl/*lpr* mice. *Eur J Pharmacol* 2002; **452**: 111–122.

81 Bessler H, Caspi B, Gavish M, Rehavi M, Hart J, Weizman R. Peripheral-type benzodiazepine receptor ligands modulate human natural killer cell activity. *Int J Immunopharmacol* 1997; **19**:249–254.

82 Camins A, Diez-Fernandez C, Camarasa J, Escubedo E. Cell surface expression of heat shock proteins in dog neutrophils induced by mitochondrial benzodiazepine receptor ligands. *Immunopharmacology* 1995; **29**:159–166.

83 Canat X, Guillaumont A, Bouaboula M, Poinot-Chazel C, Derocq JM, Carayon P, *et al.* Peripheral benzodiazepine receptor modulation with phagocyte differentiation. *Biochem Pharmacol* 1993; **46**:551–554.

84 Marino F, Cattaneo S, Cosentino M, Rasini E, Di Grazia L, Fietta AM, *et al.* Diazepam stimulates migration and phagocytosis of human neutrophils: possible contribution of peripheral-type benzodiazepine receptors and intracellular calcium. *Pharmacology* 2001; **63**:42–49.

85 Sacerdote P, Locatelli LD, Panerai AE. Benzodiazepine induced chemotaxis of human monocytes: a tool for the study of benzodiazepine receptors. *Life Sci* 1993; **53**:653–658.

86 Galiegue S, Tinel N, Casellas P. The peripheral benzodiazepine receptor: a promising therapeutic drug target. *Curr Med Chem* 2003; **10**: 1563–1572.

87 Leducq N, Bono F, Sulpice T, Vin V, Janiak P, Fur GL, *et al.* Role of peripheral benzodiazepine receptors in mitochondrial, cellular, and cardiac damage induced by oxidative stress and ischemia-reperfusion. *J Pharmacol Exp Ther* 2003; **306**:828–837.

88 Carayon P, Portier M, Dussossoy D, Bord A, Petitpretre G, Canat X, *et al.* Involvement of peripheral benzodiazepine receptors in the protection of hematopoietic cells against oxygen radical damage. *Blood* 1996; **87**:3170–3178.

89 Miccoli L, Oudard S, Beurdeley-Thomas A, Dutrillaux B, Poupon MF. Effect of 1-(2-chlorophenyl)-*N*-methyl-*N*-(1-methylpropyl)-3-isoquinoline carboxamide (PK11195), a specific ligand of the peripheral benzodiazepine receptor, on the lipid fluidity of mitochondria in human glioma cells. *Biochem Pharmacol* 1999; **58**:715–721.

90 Pelaia G, Di Paola ED, De Sarro G, Marsico SA. Is the mitochondrial benzodiazepine receptor involved in the control of airway smooth muscle tone? *Gen Pharmacol* 1997; **28**:495–498.

91 Nakajima O, Hashimoto Y, Iwasaki S. Possible involvement of peripheral-type benzodiazepine receptors in erythroid differentiation of human leukemia cell line, K562. *Biol Pharm Bull* 1995; **18**: 903–906.
92 Taketani S, Kohno H, Okuda M, Furukawa T, Tokunaga R. Induction of peripheral-type benzodiazepine receptors during differentiation of mouse erythroleukemia cells. A possible involvement of these receptors in heme biosynthesis. *J Biol Chem* 1994; **269**:7527–7531.
93 Taketani S, Kohno H, Furukawa T, Tokunaga R. Involvement of peripheral-type benzodiazepine receptors in the intracellular transport of heme and porphyrins. *J Biochem (Tokyo)* 1995; **117**:875–880.
94 Susin SA, Zamzami N, Castedo M, Daugas E, Wang HG, Geley S, *et al.* The central executioner of apoptosis: multiple connections between protease activation and mitochondria in Fas/APO-1/CD95- and ceramide-induced apoptosis. *J Exp Med* 1997; **186**:25–37.
95 Kroemer G. Mitochondrial control of apoptosis: an introduction. *Biochem Biophys Res Com* 2003; **304**:433–435.
96 Ravagnan L, Roumier T, Kroemer G. Mitochondria—the killer organelles and their weapons. *J Cell Physiol* 2002; **192**:131–137.
97 Stoebner PE, Carayon P, Penarier G, Frechin N, Barneon G, Casellas P, *et al.* The expression of peripheral benzodiazepine receptors in human skin: the relationship with epidermal cell differentiation. *Br J Dermatol* 1999; **140**:1010–1016.
98 Casellas P, Galiegue S, Basile AS. Peripheral benzodiazepine receptors and mitochondrial function. *Neurochem Int* 2002; **40**:475–486.
99 Patterson S, Spahr CS, Daugas E, Susin SA, Irinopoulou T, Koehler C, *et al.* Mass spectrometric identification of proteins released from mitochondria undergoing permeability transition. *Cell Death Differ* 2000; **7**:137–144.
100 Everett H, Barry M, Sun X, Lee SF, Frantz C, Berthiaume LG, *et al.* The myxoma provirus protein, M11L, prevents apoptosis by direct interaction with the mitochondrial permeability transition pore. *J Exp Med* 2002; **196**:1127–1139.
101 Kupczyk-Subotkowska L, Siahaan TJ, Basile AS, Friedman HS, Higgins PE, Song D, *et al.* Modulation of melphalan resistance in glioma cells with a peripheral benzodiazepine receptor ligand–melphalan conjugate. *J Med Chem* 1997; **40**:1726–1730.
102 Hirsch T, Decaudin D, Susin SA, Marchetti P, Larochette N, Resche-Rigon M, *et al.* PK11195, a ligand of the mitochondrial benzodiazepine receptor, facilitates the induction of apoptosis and reverses Bcl-2-mediated cytoprotection. *Exp Cell Res* 1998; **241**:426–434.
103 Miccoli L, Poirson-Bichat F, Sureau F, Bras Goncalves R, Bourgeois Y, Dutrillaux B, *et al.* Potentiation of Ionidamine and diazepam, two agents acting on mitochondria, in human glioblastoma treatment. *J Natl Cancer Inst* 1998; **90**:1400–1406.
104 Bono F, Lamarche I, Prabonnaud V, Le Fur G, Herbert JM. Peripheral benzodiazepine receptor agonists exhibit potent antiapoptotic activities. *Biochem Biophys Res Commun* 1999; **265**:457–461.
105 Tanimoto Y, Onishi Y, Sato Y, Kizaki H. Benzodiazepine receptor agonists modulate thymocyte apoptosis through reduction of the mitochondrial transmembrane potential. *Jpn J Pharmacol* 1999; **79**:177–183.
106 Kessel D, Antolovich M, Smith KM. The role of the peripheral benzodiazepine receptor in the apoptotic response to photodynamic therapy. *Photochem Photobiol* 2001; **74**:346–349.
107 Banker DE, Cooper JJ, Fennell DA, Willman CL, Appelbaum FR, Cotter FE. PK11195, a peripheral benzodiazepine receptor ligand, chemosensitizes acute myeloid leukemia cells to relevant therapeutic agents by more than one mechanism. *Leuk Res* 2002; **26**:91–106.
108 Jakubikova J, Duraj J, Hunakova L, Chorvath B, Sedlak J. PK11195, an isoquinoline carboxamide ligand of the mitochondrial benzodiazepine receptor, increased drug uptake and facilitated drug-induced apoptosis in human multidrug-resistant leukemia cells *in vitro*. *Neoplasma* 2002; **49**:231–236.
109 Morris RL, Varnes ME, Kenney ME, Li YS, Azizuddin K, McEnery MW, *et al.* The peripheral benzodiazepine receptor in photodynamic therapy with the phthalocyanine photosensitizer Pc 4. *Photochem Photobiol* 2002; **75**:652–661.
110 Sutter AP, Maaser K, Barthel B, Scherubl H. Ligands of the peripheral benzodiazepine receptor induce apoptosis and cell cycle arrest in oesophageal cancer cells: involvement of the p38MAPK signalling pathway. *Br J Cancer* 2003; **89**:564–572.
111 LeFur G, Vaucher N, Perrier ML, Flamier A, Benavides J, Renault C, *et al.* Differentiation between two ligands for peripheral benzodiazepine binding sites, [^{3}H]Ro5-4864 and [^{3}H]PK11195, by thermodynamic studies. *Life Sci* 1983; **33**:449–457.
112 Costantini P, Jacotot E, Decaudin D, Kroemer G. Mitochondrion as a novel target of anticancer chemotherapy. *J Natl Cancer Inst* 2000; **92**: 1042–1053.