The International Journal of Biochemistry & Cell Biology 45 (2013) 1212–1216

Contents lists available at SciVerse ScienceDirect

The International Journal of Biochemistry & Cell Biology

journal homepage: www.elsevier.com/locate/biocel

Molecules in focus

The translocator protein (TSPO): A novel target for cancer chemotherapy

Christopher J.D. Austin, Jan Kahlert, Michael Kassiou*, Louis M. Rendina**

School of Chemistry, The University of Sydney, Sydney, NSW 2006, Australia

ARTICLE INFO

Article history:
Received 21 January 2013
Received in revised form 21 February 2013
Accepted 5 March 2013
Available online 18 March 2013

Keywords:
Translocator protein
TSPO
Cancer
Apoptosis
Cell homoeostasis

ABSTRACT

The translocator protein (TSPO) is an 18 kDa transmembrane protein primarily found in the outer mitochondrial membrane where it forms a key part of the mitochondrial permeability transition pore (MPTP). Omnipresent in almost all tissues, TSPO up-regulation has been connected to neuronal damage and inflammation, making the protein an important bio-imaging marker for disease progression. More recently, TSPO has attracted attention as a possible molecular target for tumour imaging and chemotherapy. In this review we summarize TSPO's molecular characteristics and highlight research progress in recent years in the field of TSPO-targeted cancer diagnostics and treatments.

1. Introduction

The translocator protein (TSPO) was originally discovered and named the peripheral-type benzodiazepine receptor (PBR) in 1977 when investigators found a second binding site for the benzodiazepine diazepam in renal tissue distinct from $GABA_A$ receptor within the central nervous system (Braestrup et al., 1977). TSPO plays important roles in steroidogenesis, cell proliferation (in both normal and cancerous cells) and apoptosis (Papadopoulos et al., 2006).

2. Structure

The TSPO (18 kDa) is a mitochondrial protein (169 aa) encoded by nuclear DNA (*Tspo* – chromosome 22q13.31; 4 exons) and is typically located on the outer mitochondrial membrane. With 5 α-helical transmembrane domains (Fig. 1) the TSPO is believed to form a complex with several proteins of the outer and inner mitochondrial membrane collectively known as the mitochondrial permeability transition pore (MPTP), an important regulator of apoptotic and necrotic cell death during injury (Papadopoulos et al., 2006). Numerous proteins have been suggested as components of the MPTP due to their association with the TSPO (*e.g.* voltage dependent anion channel [VDAC] and adenine nucleotide transporter [ANT]), however knockout mouse studies have determined that both VDAC and ANT are non-essential for mitochondrial permeability (Baines et al., 2007; Kokoszka et al., 2004). Other proposed protein elements of the MPTP include hexokinase and creatine kinase, however the actual make-up of the MPTP remains a point of contention. Along with the TSPO, the only other confirmed component of the MPTP is cyclophilin-D, a peptidyl prolyl *cis–trans* isomerase which aids pore opening on Ca^{2+} activation and is located within the mitochondrial matrix (Woodfield et al., 1998).

The TSPO is capable of forming multimers *via* di-tyrosine bonds that covalently link individual monomers by reactions mediated by reactive oxygen species (Fig. 1, inset). The spatial arrangement of a dimeric homolog of TSPO (*Rhodobacter sphaeroides* TspO – 33.5% homology to human TSPO) was visualized using electron cryomicroscopy and single particle helical reconstruction (Korkhov et al., 2010). Described as a symmetrical dimer, each TspO monomer consists of 5 transmembrane α-helicies. Despite the low resolution (10 Å) of the 3D-structure of TspO, a glimpse of the potential architecture of mammalian TSPO within the mitochondrial membrane can be observed including two substrate translocation pathways and two potential drug-binding sites in proximity to one another. If TSPO adopts a similar configuration to TspO, experimentally observed allosterism and evidence for several different ligand binding sites (*e.g.* cholesterol binding to the cytosolic carboxy-terminus at a conserved cholesterol recognition domain [CCRD] and small molecule amino-terminus binding) can be explained by this model (Rupprecht et al., 2010).

Recently, an ortholog of TSPO, entitled TSPO2, has been discovered (Fan et al., 2009). Arising from a gene duplication event approximately 300 MYA, before the emergence of birds and

* Corresponding author. Tel.: +61 2 9351 2745.
** Corresponding author. Tel.: +61 2 9351 4781.
E-mail addresses: michael.kassiou@sydney.edu.au (M. Kassiou), lou.rendina@sydney.edu.au (L.M. Rendina).

1357-2725/$ – see front matter.
http://dx.doi.org/10.1016/j.biocel.2013.03.004

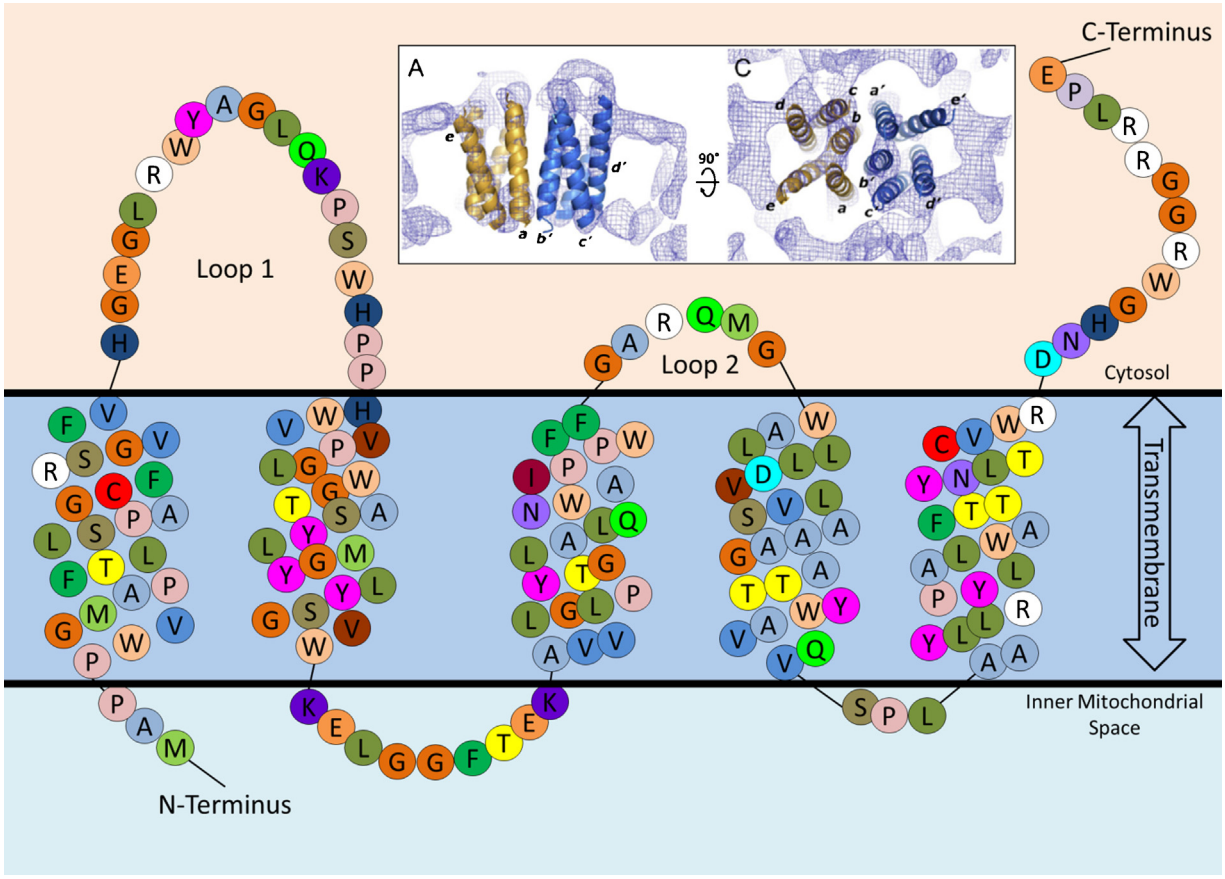

Fig. 1. *Topological model of human TSPO through the outer mitochondrial membrane.* Transmembrane helices were predicted using the TMHMM Server *v. 2.0*. Cytosolic loops 1 and 2 are responsible for the binding of small molecule ligands, such as benzodiazepines or pyrazolopyrimidines. The cytosolic C-terminal region binds cholesterol molecules. *Inset*: View of the TspO dimer, perpendicular and parallel to the membrane plane.
Reproduced with permission of the authors (Korkhov et al., 2010).

mammals, human TSPO2 shows 35% identity to human TSPO. TSPO2 is involved in cholesterol binding, transport and redistribution however it is unable to bind potent TSPO ligands, such as the benzodiazepine PK11195 (a 'gold standard' TSPO ligand). The loss of important TSPO small molecule binding amino acids (*i.e.* Arg^{24}, Glu^{29}, Arg^{32}, Leu^{31}, Leu^{37}, Lys^{39}, Pro^{40}, Trp^{107}, Val^{154} and Trp^{161}) from the extra-mitochondrial cytoplasmic loops (Fig. 1, loop 1 and 2) of TSPO2 account for this vast difference in substrate specificity. In addition, a functional human polymorphism of TSPO (rs6791 – $Ala^{147}Thr$) with a frequency of 30% within the Caucasian population has been identified (Owen et al., 2012). The TSPO-$Ala^{147}Thr$ has a lower affinity for radiolabelled benzodiazepine-like ligands than wild type protein, however the structural changes to TSPO that cause this change remain unknown, thus demonstrating that the true relationship between protein structure and metabolic activity of TSPO is poorly understood.

3. Expression, activation and substrate specificity

At the mRNA level, the TSPO is ubiquitously expressed. In both normal and abnormal tissues, the primary regulation of the *Tspo* gene expression is performed by the PKCε-ERK1/2-AP-1/STAT3 signal transduction pathway (Batarseh and Papadopoulos, 2010). As a housekeeping gene (as evidenced by multiple Sp motifs, multiple GC boxes and lack of TATA/CCAAT elements within the promoter region), TSPO is found in high levels in steroidogenic tissue and secretory glands (8–20 pmol/mg protein, *e.g.* adrenal gland, gonads, placenta). The kidney and myocardium show moderate TSPO protein expression levels (5–8 pmol/mg protein) and the brain and liver express only low levels of the protein (10–70 fmol/mg protein). Within a particular organ, distribution can vary greatly. For example, the adrenal medulla expresses no TSPO but the surrounding tissue (the adrenal cortex) expresses high levels of the protein (Casellas et al., 2002).

Within the cell, TSPO protein is most often expressed on the outer mitochondrial membrane; however other subcellular expression locations are possible. TSPO has been detected within the nuclei, lysosome, Golgi apparatus, peroxisomes and plasma membrane. Interestingly, it has also been reported that PBR/TSPO is present on mature erythrocytes, which lack both mitochondria and nuclei (Bouyer et al., 2011; Olson et al., 1988).

A wide range of endogenous ligands bind to TSPO including: cholesterol (nM affinity to CCRD), porphyrins (nM affinity) and the endozepine (diazepam binding inhibitor). As its original name (peripheral benzodiazepine receptor, PBR) suggests, the TSPO also binds to benzodiazepine-like molecules, such as diazepam, 4-chlorodiazepam, lorazepam and Ro5-4864 with moderate (μM) affinity (Nothdurfter et al., 2012). A number of other ligand classes for TSPO have been investigated including: isoquinoline carboxamides, pyrazolopyrimidines, phenoxyphenyl acetamides and aryl-oxodihydropurines (see Section 5 for further details) (James et al., 2006).

4. Biological functions

The critical nature of the TSPO to normal cellular homeostasis is evidenced by *Tspo*$^{-/-}$ which results in an embryonic lethal phenotype in mice (Papadopoulos et al., 1997). Acting as a housekeeping