

Review Article

Translocator Protein (18 kDa): A Promising Therapeutic Target and Diagnostic Tool for Cardiovascular Diseases

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The translocator protein (18 kDa) (TSPO) is a five transmembrane domain protein in mitochondria, abundantly expressed in a variety of organs and tissues. TSPO contributes to a wide range of biological processes, including cholesterol transportation, mitochondrial membrane potential and respiratory chain regulation, apoptosis, and oxidative stress. Recent studies have demonstrated that TSPO might also be involved in the physiological regulation of cardiac chronotropy and inotropy. Accordingly, TSPO ligands play significant roles in protecting the cardiovascular systems under pathological conditions through cardiac electrical activity retention, intracellular calcium maintenance, mitochondrial energy provision, mitochondrial membrane potential equilibrium, and reactive oxygen species inhibition. This paper focuses on the physiological and pathological characteristics of TSPO in the cardiovascular systems and also summarizes the properties of TSPO ligands. TSPO represents a potential therapeutic target and diagnostic tool for cardiovascular diseases including arrhythmia, myocardial infarction, cardiac hypertrophy, atherosclerosis, myocarditis, and large vessel vasculitis.

1. Introduction

The translocator protein (18 kDa) (TSPO) is a five transmembrane domain protein in mitochondria, which was previously known as peripheral benzodiazepine receptor (PBR) [1–3]. TSPO exists in a variety of species and is abundantly expressed in human organs [4–6]. It is involved in a wide range of biological processes, including controlling the synthesis of steroids, regulating mitochondrial membrane potential and respiratory chain, modulating voltage-dependent calcium channels, controlling immune response, apoptosis, and oxidative stress [1, 3, 4]. A large number of studies have demonstrated that TSPO was involved in regulating cardiac chronotropy and inotropy [4, 7–15].

Cardiovascular diseases (CVDs) are a global health issue causing tremendous economic burdens [16–18]. Considering the high abundance and significant physiological roles in the heart, TSPO has been recognized as a promising therapeutic target and diagnostic tool for CVD [7–15].

Emerging lines of evidence have suggested that TSPO plays significant roles in CVD including arrhythmia, myocardial infarction (MI), cardiac hypertrophy (CH), atherosclerosis, myocarditis, and large vessel vasculitis (LVV) [7–15]. The mechanisms responsible for its cardioprotective effects include cardiac electrical activity retention, intracellular calcium maintenance, mitochondrial energy provision, mitochondrial membrane potential equilibrium, and reactive oxygen species inhibition [1, 3, 4]. Here, we reviewed the physiological and pathological characteristics of TSPO and also summarized its role in CVD, hoping to offer a foundation for further studies on the development of TSPO as a therapeutic target and diagnostic tool for CVD.

2. TSPO

2.1. Rename. TSPO is a new name of PBR, which addressed the shortcomings and misrepresentations of PBR in the

scientific community mainly for historical reasons. Although previously this protein had multiple names known as mitochondrial diazepam-binding inhibitor (DBI) receptor complex, PK11195-binding sites, isoquinoline-binding protein (IBP), pk18, and $\omega 3$ receptor and so forth [3, 4, 7], none reflects its true nature and function. Among three main structure-function relationships for the PBR: (i) cholesterol binding and transport; (ii) protein import; and (iii) porphyrin binding and transport, transporting molecules from the outside to the inside are its major function [1, 3, 4]. The new nomenclature represents more accurately its subcellular roles and putative molecular functions. Thus, a consensus on the new name of PBR known as TSPO was reached in 2006 by the HUGO Gene Nomenclature Committee [3], which referred only to the 18 kDa protein and the minimal functional unit (binding site) of all known PBR ligands, regardless of its functional associations with other proteins [1–5].

2.2. Structure. TSPO, which consists of 169 amino acids and five transmembrane domain, can form a complex with voltage-dependent anion channel (VDAC, 32 kDa) at the outer membrane and adenine nucleotide translocator (ANT, 30 kDa) at the inner membrane of the mitochondria (Figure 1) [2, 4]. Structurally, the complex is also a combination of creatine kinase, proteins of the Bcl-2 family, PBR-associated protein 1 and protein 7. TSPO, VDAC, and ANT show a high degree of homology between various species [1]. Intracellularly, TSPO locates primarily on mitochondrial membranes, especially at the connection sites between the outer and inner membranes. Traditionally, VDAC and ANT are considered as the core components of the mitochondrial permeability transition pore (mPTP) [1]. However, a recent study exploring the effects of mammalian VDAC deletion on mitochondrial-dependent cell death proved that the wild-type and VDAC-deficient mitochondria and cells exhibited equivalent cytochrome *c* release, caspase cleavage, Ca^{2+} , and oxidative stress-induced mitochondrial permeability transition [19]. In addition, another study also showed that mitochondria lacking ANT could still be induced to undergo permeability transition. Moreover, hepatocytes without ANT remained competent to respond to various initiators of cell death [20]. Therefore, VDAC and ANT appear to be regulators rather than indispensable constituents of mPTP. Interestingly, several studies have demonstrated that TSPO may modulate the function of VDAC and ANT [1, 21, 22].

2.3. Distribution. TSPO is the product of family genes that is evolutionarily conserved from bacteria to human and exists across various species including insects, mollusks, fishes, amphibians, birds, and mammals [1, 4, 5, 23, 24]. TSPO abundantly expresses in adrenal, kidney, brain, and heart [1]. In the cardiovascular lumen, it is mainly present in platelets, erythrocytes, lymphocytes, and monocytes [1, 4]. TSPO can also be found in the walls of the cardiovascular system, such as endothelium, striated muscle, and smooth muscle [1–4, 6].

Mitochondrion is the primarily subcellular location of TSPO. However, several studies have demonstrated that TSPO could also be found in the nuclear fractions and plasma membrane [1–4]. Hence, the widespread expression of TSPO in organ, cellular, and subcellular level suggests its essential functions in biological process [1, 3, 4].

2.4. Functions. A wide spectrum of putative functions of TSPO have been suggested after binding to high-affinity ligands and cholesterol (Figure 1), such as cholesterol transportation, steroidogenesis regulation, porphyrin transportation, heme synthesis, anion transportation, cell growth and differentiation, cancer cell proliferation, apoptosis, mitochondrial membrane potential and respiratory chain regulation, voltage-dependent calcium channels modulation, and microglial activation related to brain damage and immune response [1–4, 25, 26]. However, the exact pathways regarding how TSPO is involved in those functions are still unclear [1–5, 27, 28].

Recently, a growing body of evidence indicates that TSPO ligands may play vital protective roles in cardiovascular systems through cardiac electrical activity retention, intracellular calcium maintenance, mitochondrial energy provision, mitochondrial membrane potential ($\Delta\Psi_m$) equilibrium, and reactive oxygen species (ROS) release inhibition [1, 4, 7–11]. Due to the significant clinical application potential of TSPO ligands, they represent a novel potential treatment for preventing pathological dysfunctions of CVD.

2.5. Physiological Roles in the Heart. TSPO has vital physiological roles in the heart. Accumulating lines of evidence have demonstrated that TSPO ligands, such as 1-(2-chlorophenyl)-Nmethyl- N-(1-methyl-propyl)-3- isoquinoline carboxamide (PK 11195) and 4'-chlorodiazepam (4-CIDzp), could regulate cardiac chronotropy (heart rate) and inotropy (contractile force) [29, 30]. In addition, a proposed mechanism of alteration in cardiac action potential duration and contractility has been investigated as a result of the interaction between TSPO and voltage-gated Ca^{2+} channels. TSPO is a potential pharmacologic receptor and its ligands may be essential in calcium transportation [31].

TSPO ligands, such as PK11195 which may cause adverse effects in the heart, have been identified as agonists, while ligands like 4-CIDzp that has cardiac protective effects have been known as antagonist [8, 32]. Although TSPO ligands at low concentrations seem to have no influence in heart rate, the negative chronotropy can be found at high concentrations [30, 33, 34]. PK11195 alone does not alter either inotropic effect or coronary flow velocity, while the negative inotropic effects induced by 4-CIDzp could be antagonized by PK11195 in various models including papillary muscle of guinea pigs, isolated perfused rat and rabbit hearts, and isolated canine right atrium [30, 31, 35–38].

In summary, TSPO plays significant roles in the physiological regulation of the heart. Interestingly, the negative chronotropic effects of TSPO ligand such as 4-CIDzp that have been proved in the canine [29, 39] and rabbit [37]

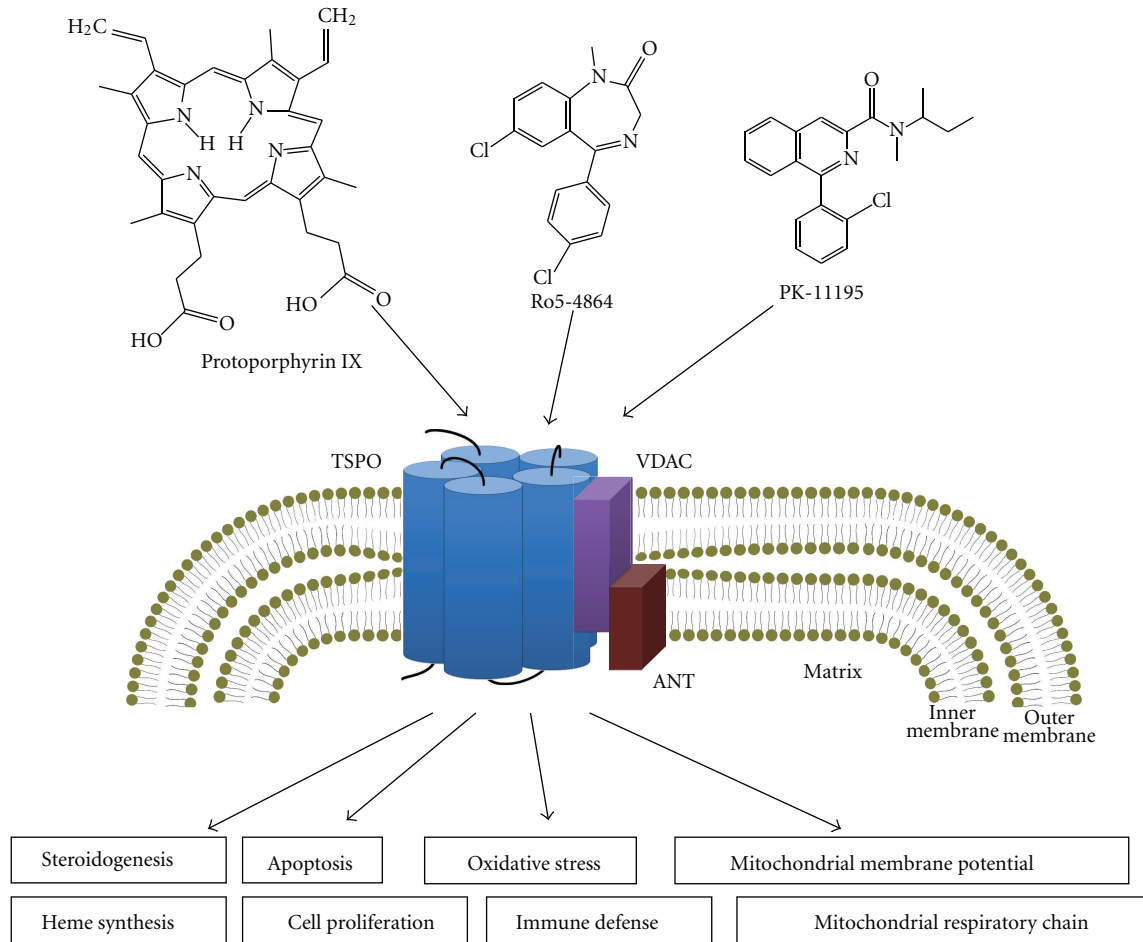


FIGURE 1: Structure and potential functions of TSPO.

cannot be identified in rat [30, 33] and guinea pig [34]. Therefore, the exact physiological role of TSPO in the heart is still unclear as great difference among species might exist.

2.6. Ligands Classification. TSPO ligands have been widely used to investigate the role of TSPO in cardiovascular diseases [1, 4, 40]. Supposed endogenous ligands of TSPO include protoporphyrin IX, diazepam binding inhibitor (DBI), triakontatetrapeptide (TTN), and phospholipase A2 (PLA2) (Figure 2(a)). Classical synthetic ligands for TSPO include 7-chloro-5-(4-chlorophenyl)-1,3-dihydro-1-methyl-2H-1,4-benzodiazepin-2-one (Ro54864) or 4-CIDzp and PK 11195 (Figure 2(b)). Novel TSPO ligands in addition to the classical synthetic ligands have also been developed, such as N,N-di-n-hexyl 2-(4-fluorophenyl) indole-3-acetamide (FGIN-1-27) and 7-chloro-N,N, 5-trimethyl-4-oxo-3-phenyl-3,5-dihydro-4H-pyridazino[4,5-b]indole-1-acetamide (SSR180575) (Figure 2(c)). Although FGIN-1-27 and SSR180575 both possess steroidogenic properties, the former is expressed as proapoptotic ligand while the latter shows antiapoptotic property [26, 41]. Recently, 3,5-Seco-4-nor-cholestan-5-one oxime-3-ol (TRO40303) (Figure 2(c)), which is identified initially for neuroprotective

properties, has been found to be a novel TSPO ligand binding specifically to the cholesterol site and exhibits the cardioprotective properties [42].

3. TSPO as a Therapeutic Target and Diagnostic Tool for CVD

3.1. Arrhythmia. Arrhythmia refers to the abnormal electrical impulses which may happen too fast, too slowly, or erratically, among which ventricular fibrillation (VF) and atrial fibrillation (AF) are most commonly reported arrhythmias associated with TSPO [43–45]. Over the last two decades, a growing body of evidence suggests that cardiac mitochondria dysfunction is a significant cause for arrhythmias [7, 8, 32, 46]. TSPO, which spans the inner and outer mitochondrial membranes, is one of the most significant targets involved in the regulation mitochondrial functions, including mitochondrial respiratory chain regulation, ROS generation and release, and inner membrane anion channels (IMACs) regulation (Figure 3) [4, 47–49].

IMAC can induce the opening of other inner membrane channels during oxidative stress and may be an effective target to prevent the metabolic oscillations [9]. Although

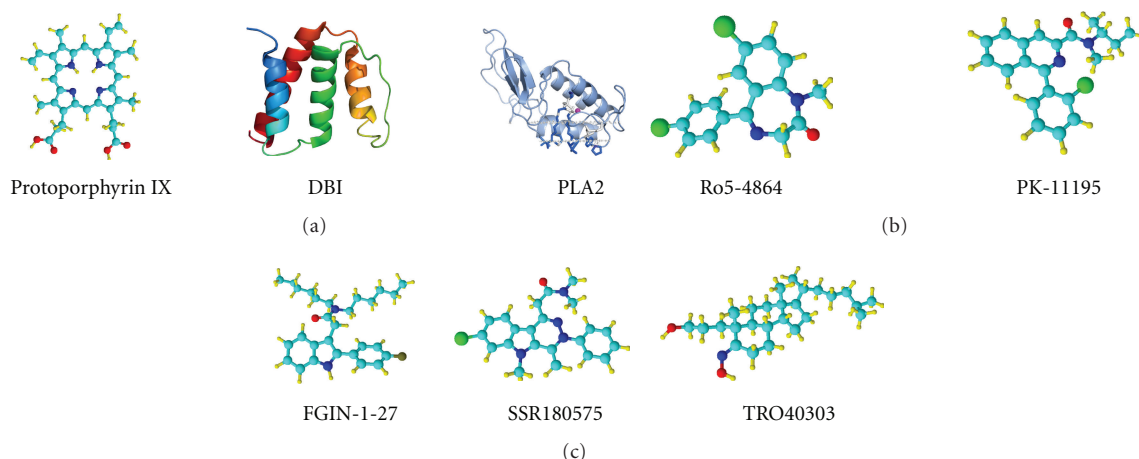


FIGURE 2: Three-dimensional structures of representative TSPO ligands. (a) endogenous ligands, such as protoporphyrin IX, diazepam binding inhibitor (DBI), and phospholipase A2 (PLA2); (b) classical synthetic ligands, such as 7-chloro-5-(4-chlorophenyl)-1,3-dihydro-1-methyl-2H-1,4-benzodiazepin-2-one (Ro5-4864) and PK 11195; (c) novel ligands, such as N,N-di-n-hexyl 2-(4-fluorophenyl)indole-3-acetamide (FGIN-1-27), 7-chloro-N,N,5-trimethyl-4-oxo-3-phenyl-3,5-dihydro-4H-pyridazino[4,5-b]-indole-1-acetamide (SSR180575) and 3,5-Seco-4-nor-cholestan-5-one oxime-3-ol (TRO40303).

the accurate structure of IMAC is not clear, the sensitivity of the anion channel regulated by TSPO ligands suggests that IMAC subunits are associated with TSPO [9, 10]. In addition, a local burst of mitochondrial ROS during ischemia/reperfusion (I/R) can lead to the increases of ROS production and oscillations in $\Delta\Psi_m$ (Figure 3), which could be blocked by TSPO ligands that have been previously known to block the activity of IMAC, such as PK11195 and 4-ClDzp [50–54]. Other investigations using different species in reperfusion-induced VF models have demonstrated that the progressive action potential shortening followed by membrane inexcitability in ischemia and VF upon reperfusion could be reduced or even eliminated by 4-ClDzp treatment before reperfusion. By contrast, FGIN-1-27 promotes $\Delta\Psi_m$ depolarization, exacerbates I/R-induced electrophysiological changes and promotes VF [8, 37, 55]. Therefore, TSPO may be an effective therapeutic target for VF.

As for AF, our group has demonstrated that inhibition of TSPO by its antagonist could significantly reduce the incidence of AF induced by ischemia-, stretch-, and cholinergic agitation [7]. In addition, the suppression of TSPO in atrial muscle cell lines could ameliorate the cytoplasm Ca^{2+} overload and energy compromise facing to chemical ischemia or cholinergic agitation [7]. Therefore, TSPO antagonists may be a novel treatment for various AF in the near future.

In summary, blocking mitochondrial IMAC through TSPO antagonists can effectively prevent mitochondrial ROS-induced ROS release and the loss of $\Delta\Psi_m$ triggered by oxidative stress [7, 8]. This effect is correlated with preservation of the action potential during ischemia as well as restoration of normal electrical activity upon reperfusion [4, 7, 47–49]. Therefore, suppression of arrhythmias, including VF and AF, by stabilization of $\Delta\Psi_m$ and inhibition of ROS overload with TSPO ligands offers us a novel therapy for arrhythmia.

3.2. Myocardial Infarction. Myocardial infarction (MI) is responsible for the majority of cardiovascular mortality. The irreversible cell injury including necrosis and apoptosis might be induced by reperfusion therapy, a major therapy for MI [56–58]. Thus, investigating a novel therapy for I/R will definitely benefit MI. I/R injury causes mitochondrial swelling and the release of cytochrome *c*, which is involved in the mPTP opening [59–62]. mPTP plays a significant role in the generation of necrotic and apoptotic cell death [59, 60]. Administration of cyclosporine A, a high-affinity inhibitor of cyclophilins that desensitizes the mPTP to the inducing effects of Ca^{2+} , can attenuate several indices of MI [60–62]. According to a study of photodynamic events mediated by porphyrins at mPTP-regulating His and Cys residues, the inactivation of His and Cys residues resulting from matrix porphyrin could be reactivated at high light dose through a different porphyrin site, which was specifically contributed by the outer mitochondrial membrane through TSPO [60, 63]. Therefore, TSPO may play a dual role in mPTP regulation including (i) as a transport protein for PTP-active compounds that are transferred to their regulatory site in the inner mitochondrial membrane or matrix and (ii) as a mPTP regulatory protein when binding to its selective ligands like porphyrins [60]. Considering the role of TSPO in mPTP regulation, it holds great promise as a therapeutic target for MI.

Our previous study has provided direct evidence that 4-ClDzp accelerated the recovery of left ventricular developed pressure (LVDP), left ventricular end-diastolic pressure (LVEDP), and maximal time derivatives of pressure measured during contraction, relaxation ($\pm dP/dt$ max) in I/R [64]. The mPTP opening is also reduced by 4-ClDzp, and thereafter a decreased ROS level could be observed [64]. Similarly, other studies have showed that 4-ClDzp reduced infarct size in a dose-dependent manner either in global or regional models of myocardial I/R in rat [65]. Thus, 4-ClDzp

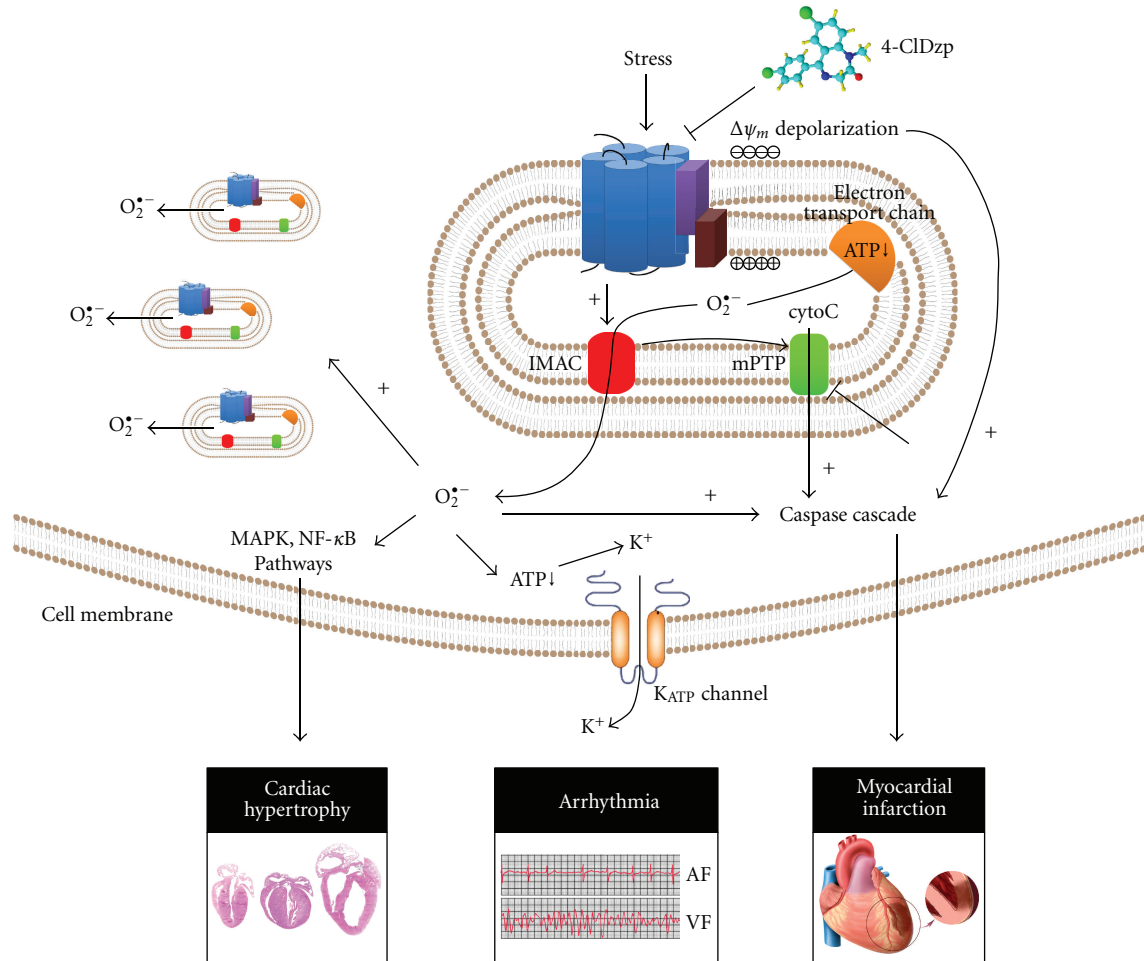


FIGURE 3: TSPO is a therapeutic target for arrhythmia, myocardial infarction, and cardiac hypertrophy. Ischemia/reperfusion rapidly induces reactive oxygen species (ROS) production from the electron transport chain, and ROS are released to mitochondrial matrix. A local burst of mitochondrial ROS leads to the increase of ROS production and oscillations in mitochondrial membrane potential ($\Delta\Psi_m$), which can destabilize neighboring mitochondrial membrane by inducing the release of ROS. The collapses of $\Delta\Psi_m$ are regulated by inner membrane anion channels (IMACs) opening, which is key in the genesis of arrhythmia. The burst of ROS in cellular matrix activates a broad variety of signaling kinases and transcription factors related to cardiac hypertrophy. The imbalance of IMAC influences the opening of mitochondrial permeability transition pore (mPTP), leading to the release of cytochrome *c* and activation of caspase cascade, and contributes to myocardial infarction. Targeting TSPO can reverse the above changes. Thus, TSPO is a therapeutic target for arrhythmia, myocardial infarction, and cardiac hypertrophy through reducing ROS.

might protect MI through reducing apoptosis, restoring mitochondrial recovery, improving oxidative phosphorylation parameters, reducing cytochrome *c* and apoptosis-inducing factor releases, and increasing the resistance of mitochondria to Ca^{2+} -induced mPTP opening [62–65].

3.3. Cardiac Hypertrophy. Cardiac hypertrophy (CH) is a common pathological feature of several major cardiovascular diseases and a physiological adaptation to increased hemodynamic overload [66–69]. Oxidative stress has been identified as one of the key contributing factors in myocardial structural damage and cardiac remodeling in CH. TSPO is associated with the regulation of cellular oxidative stress [69], which can activate a broad variety of signaling kinases and transcription factors related to CH, such as MAPK and NF- κ B (Figure 3) [66]. A strong link between oxidative stress

and extracellular matrix remodeling has been reported [66]. Besides, TSPO has been found to be associated with the protection of cells against oxygen radical damage and the regulation of mPTP opening [65, 66]. 4-ClDzp has been shown to inhibit mPTP opening and prevent isoprenaline-induced CH as a result of the reduction of heart weight to body weight ratio, left ventricular wall thickness, and myocyte size on male Wistar rats [65, 70]. Moreover, 4-ClDzp could attenuate the increase of interstitial fibrosis, lipid peroxidations, endogenous antioxidants, and β myosin heavy chain induced by isoprenaline [11]. Thus, TSPO is a potential target for CH treatment.

3.4. Atherosclerosis. Atherosclerosis is characterized with inflammatory infiltration of macrophages, dendritic cells, and activated T cells, which is initiated by lipids deposition

in the subendothelial layer of the arterial wall [71, 72]. Macrophages specifically in the atherosclerotic plaque contribute to the local inflammatory responses by secreting proinflammatory cytokines [73–75]. Activated macrophages have been demonstrated with high TSPO expression levels [76]. Several studies have investigated the uptake of [^{11}C] PK11195, a TSPO radioligand, in atherosclerotic plaques, and predicted its potential diagnostic value in atherosclerosis. They found that the uptake rate of [^{11}C] PK11195 in inflammatory regions was much higher than other areas [76, 77]. The high level of TSPO in plaque macrophages indicates a diagnostic tool through noninvasive PET imaging to predict the morphology and pathogenesis of pre-rupture atherosclerosis based on TSPO [12, 75]. Recently, the combination of ^{11}C -PK11195 PET with contrast-enhanced CT angiography offers a comprehensive assessment of plaque structure, composition, and biological activity [76, 77]. It allows the distinction between symptomatic vulnerable plaques and asymptomatic plaques with a high positive predictive value and risk stratification of asymptomatic carotid stenosis, ischemic cerebrovascular events with low CT attenuation, and high ^{11}C -PK11195 uptake [76, 77].

Besides as a potential diagnostic tool for atherosclerosis, TSPO might also be a therapeutic target for atherosclerosis. The increased ROS and decreased antioxidants have been identified in high fat and high cholesterol atherogenic (HFHC) diet rat [77]. Interestingly, the oxidative stress caused by HFHC diet was accompanied by a reduction of TSPO-binding density [77]. This finding suggested that TSPO might also be a novel therapeutic target for atherosclerosis.

3.5. Myocarditis. Myocarditis is an inflammatory myocardial disease resulting from mainly viral infections and postviral immune-mediated responses [78–80]. TSPO is abundantly expressed in mast cells and macrophages [73–77]. In order to explore the effects of TSPO in immune response to myocarditis, a study has been conducted in BALB/c mice infected with coxsackievirus B3 (CVB3) [81]. TSPO is obviously decreased in infected male mice, and the elevated levels of PLA2, an endogenous TSPO ligand, can facilitate the activation of immune cells during the innate immune response to CVB3 infection [81]. Moreover, infected male mice have a greater expression of genes which are significant in regulating the influx of cholesterol into macrophages, such as PLA2 and the macrophage scavenger receptor [81]. Therefore, increased cholesterol metabolism associated with TSPO during the immune response to CVB3 infection may drive the proinflammatory reaction of myocarditis [81, 82]. However, the exact pathways, how TSPO ligands work, remain to be investigated.

3.6. Large Vessel Vasculitis. Large vessel vasculitis (LVV) is characterized by local chronic granulomatous inflammation of the vessel wall in aorta and its main branches [83–85]. TSPO is a potential diagnostic tool for LVV because of its high expression level in macrophages which are activated

by cytokines [85, 86]. Over the past two decades, ^{11}C -(R)-PK11195 or [N-methyl- ^{11}C]-(-)-1-(2-chlorophenyl)-N-(1-methylpropyl)-3-isoquinoline carboxamide, as a radioligand specifically binding to TSPO, has been extensively used to study neuroinflammation in a wide range of brain disorders [87, 88]. Recently, a quantitative methodology of imaging LVV with ^{11}C -(R)-PK11195 deriving from both plasma and image input functions has been developed [89, 90]. This approach demonstrated that active vasculitides in patients with systemic inflammatory disorders can be quantified by macrophage targeting ^{11}C -(R)-PK11195. Moreover, the lack of significant uptake in asymptomatic patients confirmed the sensitivity and selectivity of this quantitative methodology [90]. However, further studies of reconstruction through partial-volume correction and respiration-gated acquisitions are highly needed to be conducted in a large population to minimize the vague influence of breathing on small lesions and to explore the diagnostic value of ^{11}C -(R)-PK11195 in LVV.

4. Conclusions

TSPO regulates a wide range of biological functions in cardiovascular systems. Available TSPO ligands may be utilized as therapeutic drugs or diagnostic tools for CVD, including arrhythmias, MI, CH, atherosclerosis, myocarditis, and LVV [1–3].

The visible improvements of cardiac dysfunction have been achieved by TSPO ligands [1–3, 40, 42, 45]. The cardioprotective effects are related to ROS-release inhibition, mitochondrial energy provision, cardiac electrical activity retention, and intracellular calcium maintenance [1–5, 25–28]. Although TSPO ligands could improve cardiac functions through preservation of mitochondrial physiologic effects which has been certified by a growing body of evidence, inconsistencies still exist, which might be due to different dosages and species used in studies from different groups [4, 33–39]. Therefore, future studies investigated in primate are highly needed prior to its clinical application.

In spite of the promising results from previous *in vitro* and *in vivo* studies, several limitations must be better addressed in the future. Firstly, what is the efficacy of middle-/long-term use of TSPO ligands in CVD? Secondly, are there any potential adverse effects of TSPO ligands due to the definite high expression in other tissues, such as adrenal, kidney, and brain? Finally, do TSPO ligands really offer an improved benefit-risk profile compared with current treatments or diagnosis of CVD?

Nevertheless, given the vast range of potential applications, TSPO represents a novel therapeutic target and diagnostic tool of CVD in the near future.

Authors' Contribution

X. Qi and J. Xu and F. Wang contributed equally to this work.

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