

# The Involvement of 18 kDa Translocator Protein (TSPO) in Cigarette Smoke-related Diseases: A Review

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## Abstract

The 18 kDa translocator protein (TSPO) is mainly located in the outer mitochondrial membrane, widely spread throughout the body tissues and is abundant particularly in steroid-synthesizing organs. Cigarette smoke (CS) is considered as a major risk factor for the development of oral, lung, and cardiovascular diseases, as well as cancers. TSPO protein expression is elevated in cells exposed to CS, which subsequently results in increased TSPO-related cellular processes. CS-induced overexpression of TSPO may lead to interference with cellular functioning and eventually to tissue damage, and promotes the development of various pathologies, mainly oral, lung, and cardiovascular diseases. TSPO is involved in intra-cellular functions such as apoptosis, inflammation, proliferation, and regulation of mitochondrial membrane potential. Hence, the CS-induced upregulation of the TSPO expression may contribute to the development of malignant and non-malignant oral, lung and cardiovascular diseases, including tumor growth, progression, and metastasis. Therefore, TSPO may be a target for novel treatments for various CS-associated pathologies.

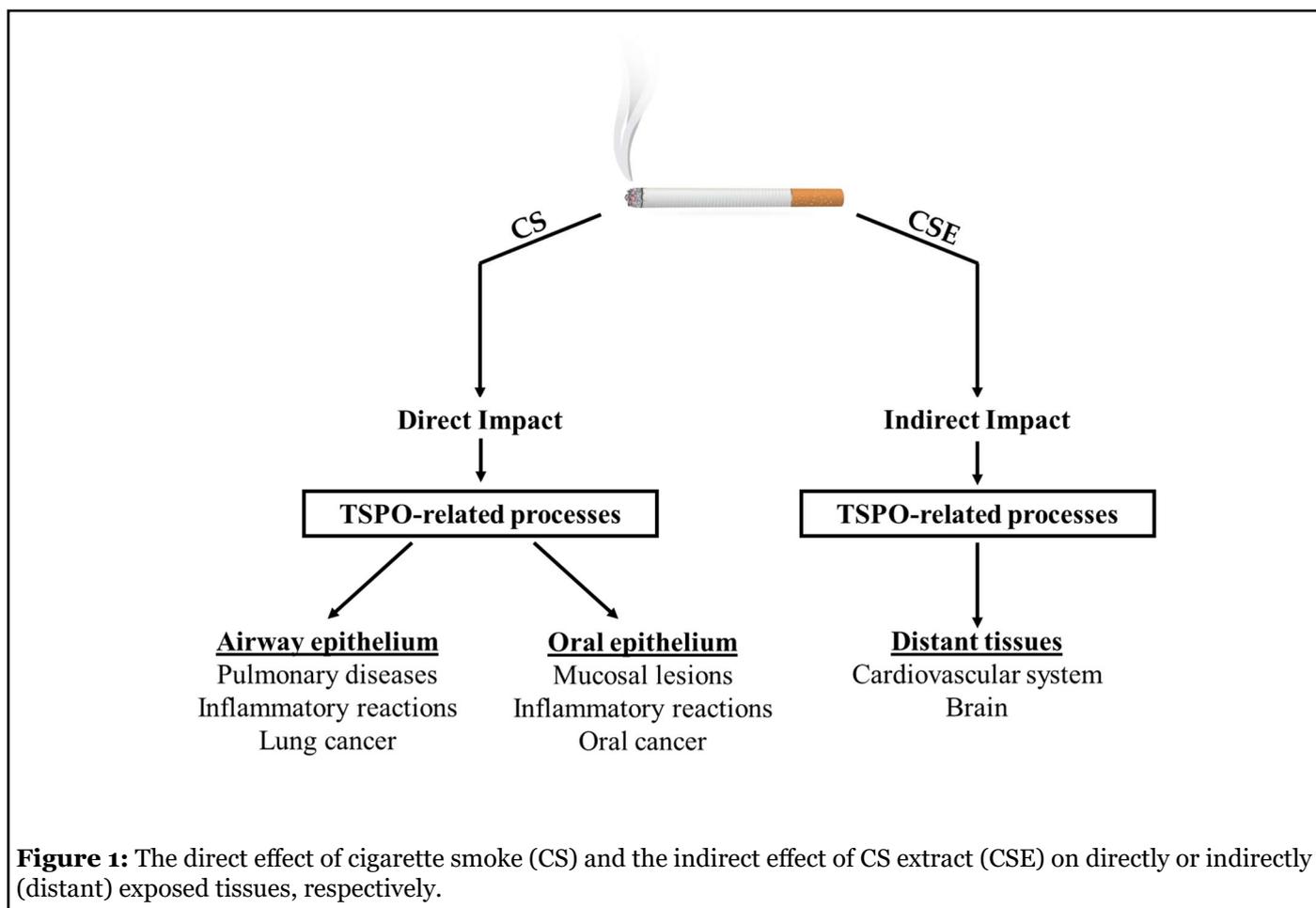
**Keywords:** Cigarette smoke (CS), Translocator protein (TSPO), Cardiovascular disease (CVD), Cancer

## Background

Cigarette smoke (CS) is a main risk factor for the development of various diseases, either via direct exposure of tissues to CS [1-3] or indirect exposure of remote organs to CS extracts (CSE) (Figure 1) [4-6]. The harmful impact is induced by exposure to the toxic CS substances, each cigarette contains approximately 7,000 chemicals. Out of these chemicals, 250 compounds are considered harmful to tissues, and 69 of them can be involved in the emergence of cancer [7].

The 18 kDa translocator protein (TSPO) was previously named as the peripheral benzodiazepine receptor (PBR), as it was first localized in rat kidneys [8], and can be found in prokaryotes and eukaryotes [9,10]. To be distinguished from the central benzodiazepine receptors (CBR), it was referred as the PBR due its distribution in peripheral tissues outside the central nervous system (CNS), and its

affinity to specific benzodiazepines (e.g. Ro5-4864). TSPO is expressed in various tissues across the body, with the highest expression in steroid-synthesizing endocrine organs [11,12], moderate expression in the kidneys and lungs [13-15], and with relatively low expression in the brain [16,17]. The TSPO name was attributed to this mitochondrial protein due to its ability to transport cholesterol across the outer mitochondrial membrane [12]. Inside the cell, TSPO is composed of five transmembrane helices across the outer mitochondrial membrane, which form a hydrophobic pocket that binds ligands at the cytosolic side of the mitochondria [18]. In the mitochondrial membrane, TSPO is found in association with the 32 kDa voltage-dependent anion channel (VDAC), and the 30-kDa adenine nucleotide translocator (ANT) [10,19]. TSPO plays an essential role in various intracellular functions including: apoptosis [10,20], cell proliferation [21-23], oxidative stress [24-26], and regulation of the mitochondrial membrane potential [26,27]. TSPO was



**Figure 1:** The direct effect of cigarette smoke (CS) and the indirect effect of CS extract (CSE) on directly or indirectly (distant) exposed tissues, respectively.

found to be involved in the pathophysiology of traumatic brain injury [28,29], cancer [17,30,31], as well as in neuroinflammation and neurodegenerative diseases [17,32,33].

An interaction was found between CS and TSPO, showing an increase in TSPO protein expression levels starting after 60 mins of exposure of H1299 pulmonary epithelial cells to CS, but not after 30 mins [3]. It is likely that the main mechanism via which CS interferes with the physiological functioning is via induction of hypoxic conditions. In the lungs, the damage associated with CS is related primarily to inflammatory response, oxidative stress and proteolysis in the pulmonary epithelial lining, eventually leading to chronic obstructive pulmonary disease [34-36]. Another study demonstrated that CS-induced damage is related to elevation in the expression of the stress response protein regulated in development and DNA damage response-1 (REDD1). Such CS-induced upregulation of REDD1 expression is a result of the CS-related hypoxia [37]. Another main target for the devastating influence of CS is the cardiovascular system. The high content of free radicals and non-radical oxidants, such as superoxide generation may lead to lipid peroxidation and eventually to oxidative damage. This pathological impact of CS is linked

with 3-fold higher risk of atherosclerotic damage to the cardiovascular system. This occurs mainly due to imbalance between the significantly increased oxidant levels and the downregulated protective antioxidants [36,38]. CS plays an essential role in the development of pulmonary diseases including chronic obstructive pulmonary disease (COPD) and cancer [2]. It was shown that a correlation exists between TSPO expression levels and the cancer aggressiveness, as upregulation of TSPO expression in cancer may modulate the activity of various cancer-related processes, such as cellular proliferation rate, angiogenesis, tumor cell migratory capability and adhesion [39]. This role of TSPO in cancer was demonstrated in different cancer types such as colorectal [25], breast [40], prostatic [41], ovarian [42] and colon [43,44] cancers.

The mechanism behind the damage caused by CS remains unclear and poorly understood [45,46]. It is possible that various mechanisms are involved in CS-related oral, pulmonary, and cardiovascular diseases [47-51]. Thus, the elucidation of the cellular and molecular mechanisms behind the occurrence of CS-related diseases is of great importance for the understanding of the pathophysiology of these diseases and the development of novel therapeutic agents.

## Relevance of TSPO to Cancer

Alterations in TSPO binding and its expression levels are involved in the development of different pathological conditions. Currently, cancers are of major concern and TSPO expression was previously demonstrated to correlate with cancer aggressiveness [39].

One of the main cellular roles of TSPO is its anti-apoptotic effect [10], which grants TSPO a protective role against cellular proliferation. Therefore, it seems that increased expression levels of TSPO aims to oppose the cancer's excessive proliferation rate, growth, and spread of metastasis [39].

An interaction between CS, TSPO expression, and oral and lung cancers was demonstrated previously [3,52-56]. This interaction is reflected by the increased expression of TSPO in CS-induced oral and lung cancer models [3,53,55]. This interaction may indicate a possible role of TSPO as a novel target for the treatment of cancer [42-44,57], including oral and lung cancers.

Tumor aggressiveness is correlated with TSPO levels, and the cellular proliferation and survival rates of animals carrying cancerous cells can be attenuated by TSPO ligands [40,58-62]. Hardwick et al. investigated the involvement of over-expression of TSPO in cancerous tissues using southern blot and in situ fluorescence hybridization analysis. They reported elevated expression of the TSPO gene in aggressive metastatic tumor cells [60]. Thus, it seems that elevated TSPO gene expression in aggressive cancers may serve as an indicator of cancer progression. In addition, a positive correlation between TSPO expression and the metastatic potential of tumors was also demonstrated in human brain gliomas and astrocytomas [22,57], as well as in colorectal cancer [63].

Based on the accumulated data on the interaction between TSPO and cancer, Veenman et al. suggested that TSPO plays a role in cell proliferation and apoptosis, since TSPO ligands inhibit cell proliferation and increased survival rate in animal models of cancer [10].

## CS, TSPO and CS-related Diseases

### CS impact on [<sup>3</sup>H]PK 11195 binding to TSPO

Our group assessed the impact of CS on the characteristics of [<sup>3</sup>H]PK 11195 binding to TSPO in various tissues and cell lines. Some studies have shown that exposure to CS is associated with decreases in TSPO binding [6,53,56,64]. It appears that exposure to CS results in a decrease in TSPO binding. As described in Table 1. Exposure of lung cancer cells (H1299 cell line) is associated with a 2-fold decrease in the [<sup>3</sup>H]PK 11195 binding following 60 mins of CS exposure [53]. Another study performed on saliva samples demonstrated a 30% decrease in [<sup>3</sup>H]PK 11195 binding as compared to saliva not exposed to CS [56]. In addition, shorter exposure times to CS was shown to decrease the binding by 75% in cardiomyocytes exposed for 30 mins to CS [6]. Similar effects of CS on TSPO binding were also detected following longer CS exposure time (90 mins). In SCC-15 tongue cancer cells, a decrease in binding levels by 72% was detected at a concentration of 3 nM of [<sup>3</sup>H]PK 11195, and by 56% at a concentration of 6 nM. In the case of SCC-25 tongue cancer cell line, a decrease in binding levels by 64% was seen at a concentration of 3 nM of [<sup>3</sup>H] PK 11195 [52] (Table 1).

It is possible that the deficient binding capacity of TSPO is involved in the development of CS-related diseases characterized by uncontrolled proliferation and growth of tissue leading to oral or lung cancer as well as to cardiovascular diseases. TSPO plays a role in cell death

Cell type	CS exposure time (mins)	Concentration of [ <sup>3</sup> H]PK 11195 (nM)	Decrease in [ <sup>3</sup> H] PK 11195 binding	Reference
Tongue epithelium (SCC-15)	90	3	64%	Nagler et al. [52]
Tongue epithelium (SCC-25)	90	3	72%	Nagler et al. [52]
Lung epithelium	60	6	2-fold	Nagler et al. [53]
Saliva	60	6	30%	Nagler et al. [56]
Cardiomyocytes	30	6	75%	Nagler et al. [6]

**Table 1:** Decreases in [<sup>3</sup>H]PK 11195 binding to TSPO following exposure of different cell types to cigarette smoke for different durations.

and apoptosis [10,65], thus, the CS-associated reduction in [<sup>3</sup>H]PK 11195 binding to TSPO may lead to uncontrolled cell proliferation.

### **Cigarette smoke impact on TSPO protein expression**

It was shown that exposure of cells to CS for prolonged time (30, 60, and 120 mins) resulted in increases in TSPO expression levels [3]. As mentioned before, in contrast to this finding, Nagler et al., reported a decrease in binding levels of the TSPO ligand [<sup>3</sup>H]PK 11195 after 30 minutes of exposure of cardiomyocytes [6] as well as in saliva samples exposed for 60 minutes to CS [56]. It is possible that the upregulation of TSPO protein expression is a consequence of its decreased binding capability. In this manner, via the elevated TSPO protein expression, the cells attempt to provide a protective response to avoid cytotoxic damage. In contrast to the finding of elevated TSPO protein expression subsequent to CS exposure, Gavish et al. demonstrated a decrease in the expression of the tetrameric 72 kDa form of TSPO following 60 mins of CS exposure of H1299 lung cancer cells [66]. Thus, it appears that there is a complex relationship between CS, TSPO binding, and TSPO protein expression.

### **Cigarette Smoke, TSPO, Oral and Lung Cancers**

CS is considered as a major risk factor for the development of oral cancer, along with other risk factors such as alcohol consumption, viral infections (mainly HPV-16 and 18), UV light and radiation [67,68]. CS is responsible for most oral cancer cases, and notably 75% of patients with oral cancer are smokers [69]. Tobacco expresses a type of synergism with alcohol, increasing further the risk for oral cancer development in alcohol users. Most oral cancers belong to the squamous cell carcinoma (SCC) family [69,70]. CS is involved also in the development of lung cancer due to the inhaled carcinogens into the alveolar spaces and the direct impact of these carcinogens on lung tissue [47]. Chronic smoking causes accumulating damages and alterations in the pulmonary tissue, and some of these damages are irreversible. The continuous irritation of the pulmonary epithelial lining might result in the appearance of pulmonary diseases such as COPD and other inflammation-related diseases, and eventually may also end in the development of lung cancer that is usually associated with high mortality rates [71,72].

The cancer-related increase in TSPO protein expression in lung cancer cell line (H1299) seems to be upregulated further following CS exposure [3]. This triad interaction (CS, TSPO, and cancer) in both the oral cavity and lung indicates the role of TSPO in CS-induced cancer in these tissues. Usually, oral and lung cancers are present in a

more aggressive form in smokers, as compared to non-smokers [69,73].

This complex association may indicate that TSPO-related pathways play a role in the development and progression of oral and lung cancer induced by CS.

### **Cigarette Smoke, TSPO, and Cardiovascular Diseases (CVDs)**

The CS associated with exposure to various oxidizing agents from the combusted cigarettes, resulting in oxidation of various molecules in various tissues throughout the body [74,75].

In the cardiac tissue, the antioxidant system prevents oxidative damage to the myocardium by opposing the production of reactive oxygen species (ROS) [76]. Several studies reported on the impact of CS on TSPO and the TSPO-related mitochondrial processes, which eventually induce oxidative stress, and may cause cell death [3,6]. This association between CS exposure and the degree of the cellular damage was shown to correlate with increased ROS production, oxidative stress, and cell death in parallel to the CS exposure time. This interaction may be involved in the increased risk of CVD, cancers, and inflammatory diseases in chronic smokers [48,76-80].

The incidence of death in myocardial infarction (MI) patients mostly occurs due to coronary heart disease (CHD), with MI proposing a risk factor for heart failure and cardiac arrhythmias [78,79]. A linear correlation described by Whincup and colleagues, demonstrated the increased risk of CHD incidence in relation to increased amount of CS exposure (number of cigarettes smoked daily) [81]. Similarly, another study showed increased relative risk of CHD incidence in association with increased duration of CS exposure in patients under 70 years of age [82]. In contrast to these linear correlations, Law and Wald described a non-linear correlation between the risk for CHD incidence and the increased amount of CS exposure. The authors suggested that the absence of the linear correlation is due to the low threshold of the effect of smoking on the risk to develop a CHD, thus display a more steady incremental fashion [49].

Penna et al. suggested that the mitochondrial permeability transition pore opening may be altered by the ischemia-reperfusion injury to cardiomyocytes, which leads to apoptosis and necrosis to commence and eventually results in MI [50]. Sensitivity to ischemia was shown to be modulated by TSPO ligands, acting as attenuators of ROS generation, which can be further modulated by activation or blockade of the inner membrane anion channel (IMAC) in the mitochondria. Such alterations attenuate the mitochondrial depolarization levels and the duration of

the action potential, imposing an antiarrhythmic impact. In this respect, several studies suggested that activation of IMAC resulted in increased sensitivity to ischemia of cardiomyocytes [76,83]. Briefly, the application of TSPO ligands was shown to possess a cardioprotective effect, via reducing ROS generation and thus decreasing the risk for arrhythmia and MI [84,85].

## Conclusions

Various studies described the putative role of TSPO and the downstream TSPO-related processes in CS-induced cytotoxic damages in association to disease development. Further comprehensive studies are warranted to clarify the underlying mechanisms in the involvement of TSPO in disease and cancer development. In addition, thorough *in vivo* investigation of the efficacy of TSPO ligands to prevent or attenuate CS-induced cytotoxicity is needed in appropriate animal models. The accumulated data may promote the development of innovative treatments of CS-induced diseases using TSPO ligands in TSPO-intact and in TSPO-knockdown cells, tissues, and transgenic mice, as well as in human clinical trials.

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