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Abstract

Increasing evidence have suggested that thiazolidinediones (TZDs) could have a therapeutic potential for patients with cancers. Here, the evidence on the mechanisms by which TZDs could contribute to different steps of cancer biology in the digestive system was summarized. According to studies, TZDs induce anti-cancer actions through 3 main pathways 1) cell growth arrest, 2) induction of apoptosis and 3) inhibition of cell invasion. Cell growth arrest is induced by increased level of $p27^{Kip1}$. $p27^{Kip1}$ accumulation is resulted from the inhibition of the ubiqutin-proteasome system and/or inhibition of MEK-ERK signaling. TZDs induce apoptosis through increased levels of apoptotic molecules, such as p53 and PTEN and/or decreased level of anti-apoptotic molecules, such as Bcl-2 and survivin. Inhibition of MEK-ERK signaling-mediated up-regulation of E-cadherin and claudin-4, and/or decreased expression of matrix metalloproteinases (MMPs) such as MMP-2 and MMP-9, play a role in the TZD-induced inhibition of cancer cell invasion. Thus, TZDs are capable of inducing anti-tumor action in a variety of ways in gastrointestinal cancers.

Key words: thiazolidinedione (TZD), anti-cancer action, digestive organs

Introduction

Thiazolidinediones (TZDs), including rosiglitazone, pioglitazone, troglitazone, and ciglitazone, are high-affinity ligands for the peroxisome proliferator-activated receptor γ (PPAR γ) [1], a transcription factor preferentially expressed in adipose tissue [2]. These TZDs improve insulin sensitivity by regulating many aspects of adipose tissue function through the transcriptional activation of certain insulin-sensitive genes involved in glucose homeostasis, fatty acid metabolism, and triacylglycerol storage in adipocytes [3, 4]. In addition to adipose tissue, many human cancer cell lines have been reported to exhibit high levels of PPAR γ expression. Exposure of these tumor cells to TZDs, especially troglitazone and ciglitazone, led to cell growth inhibition]5, 6], suggesting antitumor activities of TZDs.

In addition to basic research evidence that TZDs possess anti-tumor effects on several cultured cancer cells [5, 6], Govindarajan et al. [7] have recently demonstrated a retrospective analysis of a database from 10 Veteran Affairs medical centers in USA which was conducted to assess the influence of TZDs used to treat diabetes mellitus. The clinical study observed a 33% reduction in lung cancer risk among TZD users compared with nonusers after adjusting for confounder interactions, suggesting that

TZD use was associated with reduced risk of lung cancer. Thus, TZD clinically used is capable of preventing the development of cancers. Based on these evidence, it would be considered that TZDs have a therapeutic potential for patients with cancers.

In this review, the molecular mechanisms by which TZDs could exert anti-tumor actions in cancers in the digestive system are summarized. Studies suggest that 3 major pathways are deeply implicated in the TZD-induced anti-cancer effects. These include 1) cell growth arrest, 2) induction of apoptosis, and 3) inhibition of cell invasion, as shown in Figure 1.

Cell growth arrest by TZDs

Studies have shown that TZDs inhibit cell cycle progression at the G1/S checkpoint in cancer cells [5, 6]. So far, it has been reported increase level of cell cycle arrest-related molecules, and decreased level of cell cycle promotion-related molecules, would be involved in the TZD-induced growth arrest in cancer cells as shown in Figure 1. Among them, $p27^{Kip1}$, a cyclin-dependent kinase inhibitor, may be a key molecule that is implicated in the cell growth arrest by TZDs in human cancer cells in that TZDs increased the level of $p27^{Kip1}$ protein and the inhibition of cell

proliferation by troglitazone was not observed in cells transfected with an antisense oligonucleotide against $p27^{Kip1}$ in human pancreatic cancer cells [8]. In addition, cell growth arrest accompanied with $p27^{Kip1}$ accumulation was also observed in gastric cancer [9] and hepatocellular carcinoma [10, 11] which had been treated with TZDs, suggesting that the increased level of $p27^{Kip1}$ protein may be a common mechanism by which TZDs induce the inhibition of cell growth in a wide variety of cancer cells. Since $p27^{Kip1}$ mRNA was unaltered by TZD [11], it has been suggested that posttranslational mechanisms should have been involved in the $p27^{Kip1}$ accumulation by TZD. A ubiqutin-proteasome pathway is implicated in the posttranslational mechanisms of $p27^{Kip1}$ regulation [12-14]. The two major systems for degradation of $p27^{Kip1}$ protein, proteasome activity and function of skp2, a key enzyme for $p27^{Kip1}$ ubiquitylation as a ubiqutin ligaze' were inhibited by TZDs in human gastric cancer cells and hepatocellular carcinoma cells [9, 10, 15]. Since a proteasome inhibitor, lactacystin, inhibited proteasome activity, and increased the protein level of $p27^{Kip1}$ as well as TZD does [9], suggesting that TZD inhibits proteasome activity to accumulate $p27^{Kip1}$ protein. These results suggest that the TZD-induced growth inhibition was mediated by $p27^{Kip1}$ accumulation which is induced by both inhibition of ubiquitylation of $p27^{Kip1}$ by

down-regulation of skp2 expression and reduction of degradation activity of $p27^{Kip1}$ by proteasome as shown in Figure 1.

In addition to $p27^{Kip1}$, several molecules related to cell cycle [16] have been listed as potential mediators that are involved in cell growth arrest by TZDs. As illustrated in Figure 1, these include up-regulation of $p21^{WAF1/Cip1}$ [11] and down-regulation of cyclin D1, cyclin B1, cyclin E , CDK2 or CDK4 [17-20] .

A role of mitogen-activated protein kinases (MAPKs), extracellular signal related kinase (ERK), c-Jun N-terminal protein kinase (JNK) and p38 MAPK was examined in TZD-induced inhibition of cell growth in human pancreatic cancer cells [21] . Among the three kinases, troglitazone specifically inhibited the phosphorylation of ERK1/2 in a dose- and time-dependent manner. Troglitazone also down-regulated the protein expression of mitogen-activated protein kinase kinase **(**MEK)1/2, an upstream molecule that regulates ERK phosphorylation. Treatment of human pancreatic cancer cells with specific MEK inhibitor, PD98059 or U0126 inhibited ERK1/2 phosphorylation and cell growth. In addition, MEK inhibitors could increase expression of $p27^{Kip1}$, suggesting that the inhibition of the MEK1/2-ERK1/2 signaling pathway may be implicated in the growth inhibitory effect through accumulation of $p27^{Kip1}$ protein in

human pancreatic cancer cells. As described below, it is of interest that the inhibition of the MEK1/2-ERK1/2 signaling pathway may also contribute to the inhibition of cell invasion by TZDs (Figure 1).

Apoptosis induced by TZDs

TZDs also induce apoptosis in a variety of cells [22-25]. With regard to the mechanism by which TZDs induce apoptosis, p53 might be involved in the induction of apoptosis in gastric cancer cells as following. TZD induced apoptosis in cultured human gastric cancer cells, MKN-28, MKN-45 and MKN-74 but not in KATO-III [26]. It has been demonstrated that p53, an apoptosis-inducible gene, is deleted only in KATO-III whereas mutated or wild-type p53 is in MKN-28, MKN-45 or MKN-74, suggesting a possibility that p53 may mediate the apoptotic induction in human gastric cancer cells. BADGE, a synthetic PPAR γ antagonist [27], significantly blocked the TZDs-induced apoptosis in gastric cancer cells, suggesting that troglitazone-evoked gastric cancer cell apoptosis is mediated by PPAR γ activation. In the dominant-negative p53 mutant cells, troglitazone failed to induce apoptosis, strongly indicating p53 indeed mediates the process of the troglitazone-induced apoptosis. In addition to p53, TZDs inhibit the antiapoptotic functions of

Bcl-xL and Bcl-2 [28, 29] and stimulate apoptotic functions of bax and PTEN, leading to apoptosis [30-32] .

Survivin is the smallest member of the inhibitor of apoptosis gene family in mammalian cells [33]. High expression of survivin was associated with decreased survival in certain cancers [34]. A couple of reports have demonstrated that TZDs inhibited expression of survivin in a variety of cancer cells [35, 36]. TZD inhibits expression of survivin, thereby reducing anti-apoptotic action, in other words, inducing apoptosis. In summary, TZDs induce apoptosis through not only increased level of apoptotic factors such as p53, PTEN or bax, but decreased level of anti-apoptotic factors such as Bcl-2/Bcl-xL or survivin (Fig. 1).

In addition to the above molecular mechanisms of the induction of apoptosis by TZDs, mtDNA damage by troglitazone should be mentioned as a prime initiator of the hepatoxicity caused by this drug. Among TZDs, only troglitazone was removed from the market in 2000 because of hepatoxicity [37]. A number of hypotheses have been proposed to explain the troglitazone-induced hepatoxicity. These include the formation and accumulation of toxic metabolites, mitochondrial dysfunction and oxidant stress, inhibition of the bile salt transporter and bile acid toxicity, and the induction of apoptosis [37]. Rachek et al. [38] have recently examined

the effects of troglitazone or rosiglitazone on the primary human hepatocytes. According to the report, troglitazone induced apoptosis and significant mtDNA damage by troglitazone is considered to be a prime initiator of the hepatoxicity caused by this drug. The PPAR γ antagonist (GW9662) did not block the troglitazone-induced decrease in cell viability, indicating that the hepatotoxicity is PPAR γ -independent. Since rosiglitazone at equimolar concentrations failed to induce the change observed by troglitazone, suggesting a troglitazone-specific manner. These evidence may lead us speculate that mtDNA damage may contribute to the pharmacological actions such as apoptosis especially in cells with troglitazone.

Inhibition of cell invasion by TZDs

Increasing evidence have indicated that TZDs inhibited cell invasion and metastasis in a various kind of human cancer cells. With regard to molecular mechanisms by which TZDs exert the inhibition of cancer cell invasion, papers have been published as following. In general, matrix metalloproteinases (MMPs) or plasminogen activator inhibitor-1 (PAI-1) play a vital role in cancer cell invasion and metastasis [39, 40] . Sawai et al. [41] have demonstrated that TZD inhibited pancreatic cancer cell

invasion, which was largely mediated by modulation of the plasminogen activator system. Galli et al. [42] have shown that TZD inhibited pancreatic cancer cells invasiveness, involving MMP-2 and PAI-1 expression. According to the report by Liu et al, down-regulation of MMP-9 as well as MMP-2 by TZDs would be implicated in the inhibition of cell invasion [43] . Thus reports have suggested that plasminogen activator system and MMPs may play a role in the TZDs-induced suppression of cell invasiveness.

Epithelial-mesenchymal transition (EMT) is implicated in the progression of cancer cells [44]. E-cadherin is a key player in EMT in cancer cell progression. Recent observation has indicated that TZD increased expression of E-cadherin, suggesting that TZD up-regulates E-cadherin expression, thereby reducing cell invasive activity in human pancreatic cancer cells [45, 46]. The tight junction proteins claudins are abnormally regulated in several human cancers. Although the exact roles of claudins in tumorigenesis are still being uncovered, it is clear that they represent promising targets for cancer detection, diagnosis, and therapy [47]. For example, Michl et al. have demonstrated that claudin 4 expression decreases invasiveness and metastatic potential of pancreatic cancer [48]. Recent evidence has suggested TZD increased expression of

claudin 4 in human pancreatic cancer cells [46], suggesting that claudins expression could be changed by TZDs. It was furthermore suggested that the increased expression of claudin 4 might play a role in the TZD-induced inhibition of cell invasion in cancer cells because claudin 4 expression decreases invasiveness and metastatic potential of pancreatic cancer [48]. A MEK inhibitor, U0126, increased E-cadherin or claudin 4 mRNA and protein expression, and potently inhibited cell invasion [46]. Because TZD down-regulates MEK-ERK signaling and inhibit cell invasion in human pancreatic cancer cells [21], TZD increases expression of E-cadherin and claudin 4 possibly through inhibition of MEK-ERK signaling in pancreatic cancer cells, which might be involved in the TZD-induced inhibition of cell invasive activity (Figure 1).

PPAR-γ -independent effects by TZDs

In addition to PPAR-γ -dependent actions, TZDs demonstrate a number of PPAR-γ -independent effects [28, 49, 50]. For example, TZDs have been shown to stimulate the proteosomal degradation of cyclins D1 and D3 [51, 52] to block the cell cycle progression, and to scavenge toxic reactive oxygen species (ROS) [53] through PPAR-γ -independent

mechanisms. In addition, up regulation of PTEN/AMPK and down regulation of Akt/mTOR/p70S6 signaling cascades are involved in the anti-tumor effects by TZDs in a PPAR- γ -independent manner [50]. From a different point of view, a PPAR- γ -independent mechanism should be considered in cancer cells treated with TZDs, as following. LNCaP prostate cancer and MCF-7 breast cancer cells, both of which exhibit low PPAR γ expression levels, were more sensitive to the effects of TZDs such as troglitazone and ciglitazone on suppressing cell viability than their PPAR γ -overexpressing counterparts, PC-3 and MDA-MB-231 cells, respectively [28, 51] . Based on these PPAR- γ -independent actions of TZDs, we should consider both PPAR- γ -dependent and -independent pathways may contribute to the anti-cancer action by TZDs.

In conclusion

Investigation into molecular mechanisms that underlie TZD-induced anti-cancer effects constitutes an area of active research. Cell growth arrest, induction of apoptosis and inhibition of cell invasion seem to be deeply involved in the anti-cancer effects by TZDs. We do not know at this moment whether there are any other key molecules than listed

in this review in exerting the anti-cancer effects by TZDs. Further studies should be expected to show a better picture on the whole molecular mechanisms by which TZDs induce anti-cancer actions. Entirely understanding the molecular network related to the anti-cancer actions by TZDs could contribute to develop the treatments strategies with new perspectives.

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Figure legend

Figure 1

Schematic illustration of molecular mechanisms by which thiazolidinedione (TZD) exerts anti-tumor effects. TZD induces anti-cancer actions through 3 main pathway 1) cell growth arrest, 2) induction of apoptosis and 3) inhibition of cell invasion. Cell growth arrest is induced by increased level of cell cycle arrest-related molecules such as $p21^{WAF1/Cip1}$ or $p27^{Kip1}$, and/or decreased level of cell cycle promotion-related molecules such as cyclin D1, cyclin B1, cyclin E, CDK2 or CDK4. $p27^{Kip1}$ accumulation is induced by the inhibition of the ubiqutin-proteasome system (decreased expression of skp 2, a enzyme for $p27^{Kip1}$ ubiquitylation as a ubiqutin ligaze and inhibition of proteasome activity that degradates $p27^{Kip1}$ protein) and/or inhibition of MEK-ERK signaling. TZDs induce apoptosis through increased levels of apoptotic molecules such as p53, PTEN or bax, and/or decreased level of anti-apoptotic molecules such as bcl-2/bcl-xL or survivin. Inhibition of cell invasion is induced by TZDs through up-regulation of E-cadherin and claudin-4 and/or down-regulation of MMP-2, MMP-9 or PAI-1. Inhibition of MEK-ERK signaling by TZDs may be involved in the increased expression of E-cadherin and claudin-4.

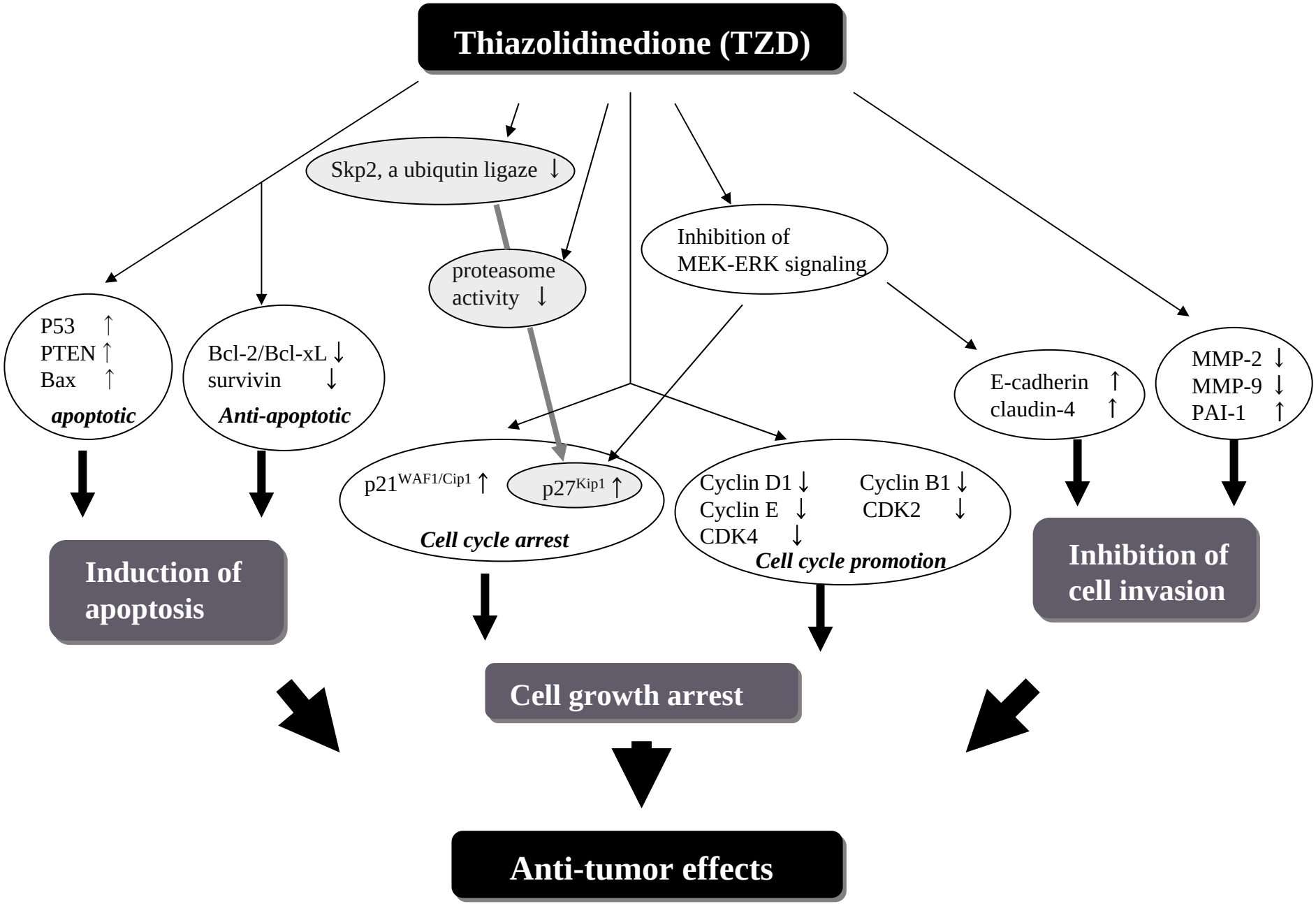
Thiazolidinedione (TZD)
Skp2, a ubiqutin ligaze ↓
proteasome activity ↓
Inhibition of MEK-ERK signaling
P53 ↑
PTEN ↑
Bax ↑
apoptotic
Bcl-2/Bcl-xL ↓
survivin ↓
Anti-apoptotic
p21WAF1/Cip1 ↑
p27Kip1 ↑
Cell cycle arrest
Cyclin D1 ↓
Cyclin B1 ↓
Cyclin E ↓
CDK2 ↓
CDK4 ↓
Cell cycle promotion
E-cadherin ↑
claudin-4 ↑
MMP-2 ↓
MMP-9 ↓
PAI-1 ↑
Induction of apoptosis
Inhibition of cell invasion
Cell growth arrest
Anti-tumor effects

Fig 1