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TUDCA receptors and their role on pancreatic beta cells

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ABSTRACT

Bile acids have received increasing attention over the past years as their multiple alternative roles became clearer. Tauroursodeoxycholic Acid (TUDCA) in specific has generated special interest due to its ability to promote pancreatic survival and function, as well as reduce endoplasmic reticulum stress. However, there are few studies explaining the molecular mechanisms behind TUDCA's beneficial actions on pancreatic beta cells. In this review, we decided to review the literature in order to craft a primer for researchers on what is known about TUDCA's receptors and the molecular pathways involved in this bile acid's function in the endocrine pancreas. We review the studies that focused on G protein-coupled bile acid receptor (TGR5), Sphingosine-1-phosphate receptor 2 (S1PR2) and α5β1 Integrin function in pancreatic cells. Our hope is to provide a basis for future studies to expand upon, especially considering the current lack of studies focusing on the importance of these receptors, either through TUDCA signaling or other signaling molecules.

Contents

1. Introduction

The Tauroursodeoxycholic Acid – more known by its acronym TUDCA – is a secondary bile acid; it is the taurine-conjugated form of Ursodeoxycholic acid (UDCA) (Vang et al., 2014). Initially used for the treatment of liver diseases, TUDCA showed many unexpected uses for the treatment of neurodegenerative diseases, cardiovascular diseases, gastrointestinal dysfunctions, glucose metabolism as well as other functions, as previously summarized in other papers (Vang et al., 2014; Zangerolamo et al., 2021; Cortez and Sim, 2014).

Most importantly, TUDCA showed immense promise in the treatment of metabolic diseases like obesity and diabetes. For

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example, TUDCA improves liver and muscle insulin sensitivity in humans by acting on G-protein-coupled receptors that promote phosphorylation of downstream targets such as Protein Kinase B (Akt) and Insulin Receptor Substrate 1 (IRS) (Kars et al., 2010). TUDCA also inhibits adipose tissue inflammation in high-fat diet mice (Chen et al., 2016). TUDCA's ability to combat endoplasmic reticulum (ER) stress - a well-documented property of this bile acid, which improves protein folding by acting as a chemical chaperone - also plays a role in these effects.

On top of improving metabolic parameters like insulin sensitivity, TUDCA can also protect pancreatic beta cells from dysfunction and apoptosis. The co-treatment of INS-1E pancreatic beta cells line with toxic concentrations of palmitate and TUDCA showed that the bile acid was capable of reducing both apoptosis and growth inhibition of those cells (Zhu et al., 2013). This protection was also observed *in vivo* in pre-diabetic mice, which received 250 mg/kg of TUDCA for 16–30 weeks (Engin et al., 2013).

It is known that TUDCA treatment increases glucose-stimulated insulin secretion (GSIS) in isolated islets from mice, improving the overall function of the pancreatic beta cell (Vettorazzi et al., 2016). In addition, the potentiation of GSIS by TUDCA was also observed in isolated islets from a mouse model of Alzheimer's disease (Zangerolamo et al., 2020). Remarkably, the treatment with TUDCA has also been shown to increase the number of beta cells in pancreatic islets (Zangerolamo et al., 2020; Bronczek et al., 2019), through as yet unknown mechanisms.

In view of that, the full scope of TUDCA's mechanisms of action is still not fully understood. One of the reasons that many studies do not address the receptors and mechanisms involved in the actions of TUDCA is the wide variety of receptors and molecular pathways that can be activated by TUDCA and other bile acids. Bile acids can act on both intracellular and membrane receptors (McMillin and DeMorrow, 2016; Daruich et al., 2019). The main membrane bile acid receptors currently known are the G protein-coupled bile acid receptor 1/Takeda G protein-coupled receptor 5 (GPBAR1/TGR5), Sphingosine-1-phosphate receptor 2 (S1PR2) and α5β1 Integrin. Meanwhile, the main intracellular receptors that interact with bile acids are Farnesoid X receptor (FXR), Vitamin D Receptor (VDR), Pregnane X receptor (PXR), Glucocorticoid receptor (GR) and Constitutive Androstane Receptor (CAR) (McMillin and DeMorrow, 2016; Daruich et al., 2019). Of these, TUDCA has well-established interactions with TGR5 (Yanguas-Casas et al., 2017; Dicks et al., 2020; Wu et al., 2020), S1PR2 (Vettorazzi et al., 2017; Studer et al., 2012) and α5β1 Integrins (Daruich et al., 2019; Gohlke et al., 2013; Häussinger et al., 2003) in multiple cell lines, to exert multiple functions. The affinity of the main unconjugated bile acids, as well as their derivatives, which act as agonists for these receptors, has been previously summarized (Wan and Sheng, 2018). The relationship of TUDCA with FXR is a bit more controversial, and will be discussed in greater detail in its own section within this paper.

Thus, we will review TUDCA's interactions with these established receptors (TGR5, S1PR2, α5β1-integrins), as well as the controversial nature of FXR, and the consequences of those interactions, with an emphasis on the pancreatic beta cell – its ability to protect and maintain the pancreatic beta cell function. Our hope is to form a clearer picture of the elements involved in this process. This is of great interest for the scientific community due to TUDCA's potential as a therapeutic adjuvant for metabolic diseases. The literature of TUDCA's effects on pancreatic beta cells is scarce, and so is the literature of the effect of the mentioned receptors, making it even more important to review the known information to raise perspectives that will provide a jumping point for future studies.

2. Receptors and their roles

2.1. TGR5

One of the most important TUDCA receptors, TGR5 is a G-protein coupled receptor (Guo et al., 2016). It was the first G-protein coupled bile acid receptor discovered, back in 2002, and is coupled with a $G_{\alpha s}$ protein. Its activation leads to an increase in intracellular cAMP, which then triggers a signaling cascade that leads to its effects (Guo et al., 2016). TGR5 has a relationship with important downstream signaling pathways, such as NF-κB, Akt and Extracellular signal-regulated kinases 1/2 (ERK1/2) (Guo et al., 2016). TGR5 activation has a myriad of metabolic effects; for example, it leads to a reduced body weight in high fat diet (HFD)-fed mice, due to an increase in energy expenditure directly related to TGR5-activation in mice brown adipose tissue, and myoblast activation in humans (Pols et al., 2011). It also improves glucose metabolism, which seems to be the result of increased Glucagon-like Peptide 1 (GLP-1) secretion dependent of TGR5 activation; the use of a specific TGR5 agonist led to increased GLP-1 secretion in both mice and human intestinal enteroendocrine cell lines (Pols et al., 2011). TGR5 activation also has protective effects in the liver, reducing steatosis and damage to the hepatocytes (Pols et al., 2011).

TGR5 also plays an important role in the pancreas – and, specifically, in the pancreatic beta cell. On the beta cell itself, TGR5 receptors seem to mainly play a role in insulin secretion. The first evidence of this was found by Kumar et al. they observed that treatment with a selective TGR5 ligands in MIN6 cell line, as well as in human and mice isolated islets, resulted in an increase of insulin secretion in a manner dependent of G_s/cAMP/Ca^{2+} (Kumar et al., 2012). Kumar et al.'s data proposed that the mechanism was PKA-independent; however, two future studies reinforced the TGR5 role in promoting insulin secretion through a cAMP pathway, but also implied Protein Kinase A (PKA) in the process (Vettorazzi et al., 2016; Maczewsky et al., 2019). The first study did so by utilizing isolated islets from mice; TUDCA promoted glucose-stimulated insulin secretion, but its ability to do so was disrupted when cells were treated with a cAMP or a PKA inhibitor, highlighting the importance of both (Vettorazzi et al., 2016). The second study also used PKA inhibitors to measure how they would affect the response of pancreatic beta cells to oleate, a different substance that also activated TGR5. Indeed, they found that PKA inhibition led to a decrease of the TGR5-mediated increase of insulin secretion (Maczewsky et al., 2019). There is also an important cross-talk between alpha and beta cells that is mediated by TGR5 activation; the use of a TGR5 agonist led to a switch from glucagon to GLP-1 production by the alpha cells under hyperglycemic conditions, due to increased activation of PC1 in these cells, which consequentially improved beta cell survival and function due to the paracrine effect of the released GLP-1 (Kumar et al., 2016).

As such, it seems safe to assume that PKA plays an important role in TGR5's action on the pancreatic beta cell. This would be in line with studies in other cell types that have pointed to an important cAMP-PKA signaling mechanism when this receptor is activated by ligands, such as TCDCA to promote anti-inflammatory roles in lung cells and macrophages (Qi et al., 2020), and INT-777 in neuronal cells (Hu et al., 2021). Furthermore, it has been shown that TGR5 activation by TUDCA activates the cAMP-PKA molecular pathway in microglia cells, an effect involved, at least in part, with the anti-inflammatory effects exerted by this bile acid on these cells (Yanguas-Casas et al., 2017). These consistent anti-inflammatory effects point to a potential effect of TGR5 activation on the protection of pancreatic beta cells against potentially harmful stimuli; in fact, this is further supported by delving deeper into TGR5's mechanisms of action in other cell types. For example, as previously

mentioned, TGR5 signaling has important relations with pro-survival elements like ERK1/2 (Dent et al., 2005), stimulation of Akt/mTOR signaling and inhibition of NF-κB (Guo et al., 2016), all of which play important roles in pancreatic beta cell survival (Costes et al., 2006; Srinivasan et al., 2002; Melloul, 2008). So far, however, studies on pancreatic beta cells have focused on TGR5's ability to improve cellular function far more than on its potential effects on cellular survival. This could be a worthy avenue to pursue in future studies on the subject.

2.2. S1PR2

S1PR2, like TGR5, is also a G-protein coupled receptor; however, S1PR2 is coupled to a $G_{\alpha i}$ and $G_{\alpha Q}$ proteins instead (Chiang, 2015; Adada et al., 2013). It is one member of a family of 5 S1P receptors. The activation of S1PR2 has been linked to endothelial cell function (Chiang, 2015), regulates inflammation (Adada et al., 2013), and has a pretty established role in cancer, with both pro and anti-tumor growth effects depending on the cell type (Adada et al., 2013). It has been shown to possess important metabolic functions for both the pancreas (explained below) and the liver. In the liver, it modulates lipid metabolism (Nagahashi et al., 2015) as well as glucose homeostasis and bile acid synthesis (Adada et al., 2013). Akt and ERK1/2 were identified as the downstream targets responsible for these actions.

In the pancreas, S1PR2 plays an interesting and somewhat puzzling role in pancreatic beta cell survival. Imasawa et al. showed that the blockade of S1PR2 signaling, both through deficiency of the receptor in genetically-engineered mice and through the use of selective antagonists in wild-type mice, protected beta cells from streptozotocin-induced apoptosis (Imasawa et al., 2010). A later study conducted in 2015 also observed that treatment with a S1PR2 antagonist protected HFD-fed, New Zealand Obese (NZO) mice beta cells from damage, reinforcing the finding that S1PR2 inhibition promotes beneficial effects for the cell (Japtok et al., 2015).

The role of S1P in the beta cell seems to be complex and multifaceted; it usually acts as a pro-survival molecule but, in high concentrations, it can counteract insulin's pro-survival effect on beta cells, resulting in a pro-apoptotic action. This seems to be mediated specifically by S1PR2, rather than other S1P receptor subtypes (Japtok et al., 2015). There were also studies using FTY720, an agonist of S1P receptors except S1PR2, that showed that treatment with this agonist resulted in a decrease in the incidence of diabetes in mice, indicating that S1PR2 is not a necessary element of S1P's beneficial effects on beta cells (Maki et al., 2005).

In a 2019 study *in vivo*, the researchers treated HFD-fed mice with S1P and evaluated the effects of the treatment on parameters such as cell proliferation and apoptosis. They observed that S1P treatment did improve those parameters, increasing proliferation and reducing apoptosis, and that S1PR2 expression was (together with S1PR1) increased in the HFD and HFD + S1P groups (He et al., 2019). This led them to conclude that S1PR2 activation could be a possible mechanism through which S1P exerted its positive effects on pancreatic beta cells. However, no deeper molecular experiments were done, only the expression of receptors detected by immunostaining, which makes it harder to consider this finding as a refutation of the previously-established findings of the antagonism of S1PR2 being beneficial to the cell instead.

This overall picture – of S1PR2 blockade being beneficial for the cell - does seem paradoxical with TUDCA's effects; TUDCA promotes survival in the beta cell, and S1PR2 is one of its receptors, as previously established. Thus, either TUDCA somehow does not interact with S1PR2 in the beta cell, or this interaction does not result in a pro-apoptotic outcome. While this is speculation, it is possible that TUDCA's interaction with the other two receptors counteracts the pro-apoptotic effect of S1PR2, leading to a net anti-apoptotic outcome, or some sort of cross-talk between the other receptors and S1PR2 inhibits the usual pro-apoptotic effect of its activation. It is also possible to imagine that TUDCA acts as an antagonist, rather than an agonist, for S1PR2 in the beta cell.

Unfortunately, there does not seem to be studies utilizing TUDCA in the beta cell that discuss potential roles of S1PR2, which makes it hard to discuss these speculative hypotheses. As such, we turn to non-pancreatic studies for insights. In hepatocytes, blockade of S1PR2 signaling through specific antagonist JTE-013 resulted in an inhibition of ERK1/2 and Akt signaling in response to TUDCA treatment (or treatment with other bile acids and with S1P) (Studer et al., 2012). Researchers proposed that S1PR2 activation is an important mechanism of the beneficial effects of conjugated bile acids such as TUDCA in the prevention of apoptosis in hepatocytes (Studer et al., 2012). Another interesting finding about the effects of TUDCA mediated by S1PR2 was reported by Vettorazzi and collaborators (Vettorazzi et al., 2017). In this study, TUDCA increased insulin-degrading enzyme (IDE) expression in human hepatic cell line HepG2, by an S1PR2-IR-PI3K-Akt pathway dependent mechanism; once in the presence of the S1PR2 inhibitor, JTE-013, TUDCA failed to increase the expression of IDE. These findings suggest that TUDCA is involved not only with beta cell function and survival, but also with insulin metabolism.

There are also reports of a pro-apoptotic role of S1PR2 activation against hepatotoxic bile acids (GCDC, in the case of this study) (Webster and Anwer, 2016). An interesting finding of this study is that this pro-apoptotic effect is not due to the direct ligation of the bile acid to S1PR2 but instead with S1PR2 being activated by S1P generated as a response to the entry of GCDC within the cell. This S1P ligand would activate the $G_{\alpha i}$ protein which would result in lower cAMP production, lower Akt activation, and contribute to apoptosis (Webster and Anwer, 2016). Elevated levels of cAMP has been shown to promote survival in pancreatic beta cells (Jhala et al., 2003), so if the receptor's mechanisms works in a similar manner in the beta cell, this would give credence to our proposed hypotheses – for example, TUDCA may impede S1P from activating S1PR2's effects over cAMP, or its mutual activation of TGR5 (pro-cAMP) and S1PR2 (anti-cAMP) results in a net-positive effect for the cell. In terms of mechanisms, S1PR2 seems to be another way through which TUDCA may modulate elements such as ERK1/2 and Akt, much like TGR5. However, these are only some possible ideas, and further studies investigating TUDCA's effect on S1PR2 specifically within the beta cell are needed.

2.3. α5β1-integrins

Integrins have long been recognized as important receptors for cellular adhesion and for the signaling of a multitude of processes, including proper embryonic development, cell and tissue survival, cell migration and tumor angiogenesis (Harburger and Calderwood, 2009; Rocha et al., 2018). They are a large family with multiple subunits – specifically, 18 α and 8 β subunits (Rocha et al., 2018), each of them being a transmembrane protein. Integrin signaling involves conformational changes of protein complexes on the internal, or cytoplasmic, end of the integrins (Harburger and Calderwood, 2009). Integrin signaling requires activation by talin, which interacts with the cytoplasmic tail of the β subunit. This leads to conformational changes that increase the affinity of the receptor to its ligands (Harburger and Calderwood, 2009), and protein complexes assembly. This complex interacts with multiple other proteins, such as Focal Adhesion Kinase (FAK), Paxilin, Integrin-linked Protein Kinase (ILK) and others (Harburger and Calderwood, 2009), which will then promote a signaling cascade of protein interactions that will result in the observed effects

(Harburger and Calderwood, 2009).

α5β1-Integrins can also act as receptors for bile acids, with a specific affinity for TUDCA (Gohlke et al., 2013), which is the only bile acid currently known to activate these receptors (McMillin and DeMorrow, 2016). There are many studies showing the importance of β1-Integrins in the beta cell; not only during pancreatic development (Wang et al., 2005; Yashpal et al., 2005), but also during the adult life; for example, β1-Integrin blocking results in decreased insulin expression and an increase in apoptosis, on top of cell adhesion problems, in human cells (Wang et al., 2005). This was also observed in-vivo; β1-Integrin deficiency in mice led to decreased insulin content, beta cell mass and proliferation, as well as impaired glucose tolerance (Riopel et al., 2011). Riopel et al.'s study also noticed a decrease in FAK and ERK1/2 phosphorylation, which led them to conclude that β1-Integrin signaling happens through a FAK-Mitogen-Activated Protein Kinase (MAPK)-ERK pathway (Riopel et al., 2011). A posterior study also reinforced these results and the overall importance of β1-Integrin signaling for beta cell function (Peart et al., 2017). In it, researchers utilized a β1-knockout mice model and observed that this deficiency led to problems in insulin secretion and reduced islet beta cell mass, with reduced FAK, ERK1/2 and Akt signaling in the β1-integrin deficient mice. As such, β1-Integrin signaling seems to be crucial for proper beta cell function and survival, and α5β1-Integrin might be a very important receptor for TUDCA's action on the beta cell. However, while there is some evidence of β1-Integrin importance, we did not find any studies focusing specifically on α5β1-Integrins in the beta cell, which means we cannot conclusively discuss any potential particularities of this subtype. Considering this subtype has already been identified in the beta cell (Krishnamurthy et al., 2008) and its specificity as a TUDCA ligand, future studies focusing on it could be of great interest.

Once again, there are essentially no papers discussing mechanisms of action of TUDCA and α5β1-Integrins in the beta cell, or even mechanisms of these receptors as a whole. As such, once more it is important to turn to other cell types for insights. In hepatocytes, the relationship between TUDCA and α5β1-Integrins is well-established (Gohlke et al., 2013; Beuers, 2013). Interestingly, TUDCA's ability to activate α5β1-Integrins in these cells seems to be dependent on cell entry, exerting its effects within the cytosol rather than at the cell membrane (Gohlke et al., 2013). This entry into the cell seems to be mediated by NTCP, a hepatocyte-specific solute carrier, and TUDCA's ability to activate α5β1-Integrins was lost in hepatocytes cell lines that do not express NTCP (Gohlke et al., 2013). The downstream signaling elements implied include FAK, PI3K and MAPK (Gohlke et al., 2013). This opens up a few unsolved questions for the understanding of TUDCA's action in the pancreatic beta cell – namely, if cell entry is also necessary for TUDCA to exert effects on α5β1-Integrins in these cells and, if yes, through which mechanisms and/or carriers this entry is done. Likely this will include FAK signaling and downstream elements such as ERK1/2, PI3K and Akt, but concrete evidence is still lacking. The question of TUDCA entry into beta cells is also relevant for the next topic.

2.4. FXR

FXR is one of the most important bile acid receptors; indeed, it was the discovery of FXR's interaction with bile acids that kick-started the topic of bile acids as cell signaling molecules (McMillin and DeMorrow, 2016). It plays important roles in the liver and gut, regulating the levels of bile acids through feedback mechanisms, and its inhibition *in vivo* perturbs the metabolism of lipoproteins, as recently reviewed (Ferrell and Chiang, 2021).

However, its involvement with TUDCA, and the importance of this interaction, seems to be controversial within the literature. Some studies point out that FXR has a low affinity for TUDCA (and its precursor, UDCA) (Liu et al., 2003). The proposed reason for this is TUDCA's hydrophilic nature that prevents it from entering the cell and thus interacting with an intracellular receptor like FXR (Chiang, 2013; Sepe et al., 2014). However, a 2018 study has shown that TUDCA seems to have a surprising antagonistic effect over FXR both *in vivo* and in vitro (Sun et al., 2018). And a later study showed that TUDCA seems to upregulate SIRT1-FXR signaling in the liver of rats under hemorrhagic shock conditions, as well as in vitro HepG2 cells under hypoxic conditions (Sun et al., 2020). It seems that TUDCA can possibly exert its functions on FXR if the cell type possesses a bile acid transporter, such as NTCP (Kiriyama and Nochi, 2019). This, indeed, would solve the problem created by TUDCA's hydrophilicity, although whether this interaction results in agonism, antagonism, or varies from cell type still needs elucidation.

The role of FXR in the pancreatic beta cell has been previously reviewed (Düfer et al., 2012). In short, it has been shown to exist in beta cells of human and animal origin, and its activation leads to an improvement of beta cell function, especially to insulin secretion (Düfer et al., 2012). However, in regard to TUDCA, we failed to find any studies that showed TUDCA promoting effects in the pancreatic beta cell through FXR. The only relevant finding was in Vettorazzi' et al., 's 2016 work (Vettorazzi et al., 2016), in which co-treatment of TUDCA and tauro β-muricholic acid (TβMCA), an FXR antagonist, did not affect TUDCA-induced insulin secretion.

It is possible that FXR is part of TUDCA's actions on the beta cell, but it seems unlikely in the face of current evidence. It would require the existence of an adequate carrier to transport TUDCA within the cell, or some other mechanism for TUDCA internalization within these cells – and even then, affinity may be too low to have a relevant effect. To our knowledge, this mechanism of entry has not been found so far. The same goes for other intracellular bile acid receptors such as VDR and GR.

3. Conclusion and perspectives

In conclusion, the TUDCA receptors all play important roles in pancreatic beta cell survival and function (Fig. 1). However, all of them have been the target of just a handful of studies, and further understanding of their signaling pathways is required. Specifically, TGR5 seems to be the main mediator of TUDCA's improvements over beta cell function, especially in regards to insulin secretion, although α5β1-integrins also seems to play a role; meanwhile, its pro-survival effects seem to be mediated by all three receptors, indirectly by TGR5 due to paracrine GLP-1 secretion from alpha cells, and directly by α5β1-Integrin activation and S1PR2 (and possibly TGR5 too, as many of its downstream elements are pro-survival). α5β1-Integrin, specifically, seems to be overlooked at the moment, with most studies focusing in β1-Integrins in general rather than the α5 variety in specific. The underlying molecular mechanisms of α5β1-Integrin signaling should be explored more deeply.

In terms of perspectives for the future, our review of the literature raised more questions than answers, which points to the potential of the subject as a research topic. Notable questions that still remain to be answered, in our opinion, include: TGR5's potential role in pancreatic beta cell survival; the paradoxical effects of S1PR2 and how TUDCA still performs a beneficial role to the cell when interacting with it (some hypotheses worth investigating: TUDCA acting as an antagonist rather than agonist for S1PR2 in the beta cell; TUDCA's activation of other receptors alongside S1PR2 negates S1PR2's pro-apoptotic effects or results in a net positive for the cell); the question of whether TUDCA is internalized in pancreatic beta cells (and if yes, does it have a relevant interaction with intracellular receptors such as FXR? If not, is α5β1-integrin

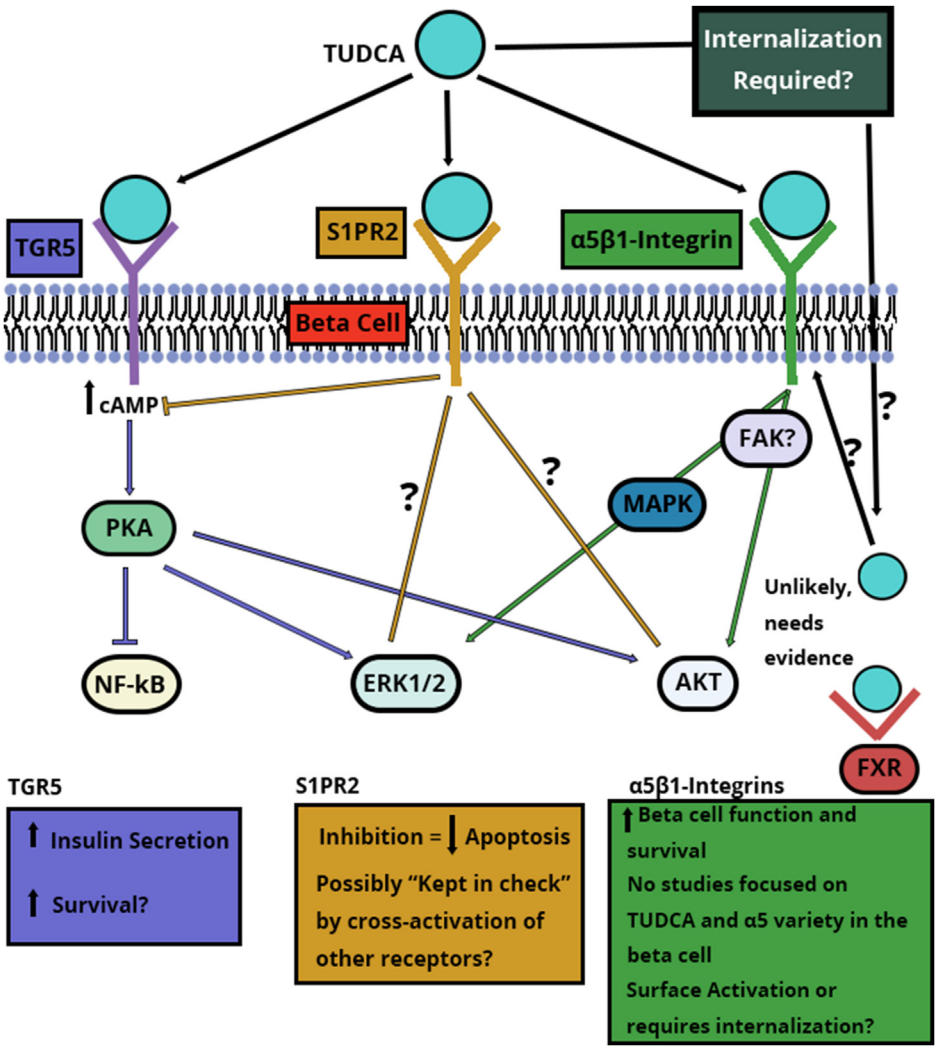

Fig. 1. Illustrative representation of the three main TUDCA receptors and their main roles in the pancreatic beta cell, as currently known in the scientific literature, as well as possible underlying mechanisms. TGR5 plays an important role in glucose-stimulated insulin secretion. S1PR2 inhibition improves beta cell survival. And α5β1-integrins seem to play an important role in beta cell function and survival. Many questions remain open, notably regarding S1PR2's and α5β1-integrin's mechanisms.

independent of this internalization in the beta cell?); how relevant is α5β1-integrin signaling for TUDCA's effects on pancreatic beta cells; as well as general elucidations on downstream signaling pathways and how – and if – they differ from TUDCA's signaling in other cell types.

Our review provides an overview of TUDCA receptors and their beneficial effects on the beta cell. However, further studies are still needed to explore these receptors and the molecular pathways that mediate the therapeutic effects of TUDCA on pancreatic beta cells, as well as unravel new roles for this bile acid, that will be important for translation to the clinical setting. Currently, there is only one registered clinical trial in progress with TUDCA in Type 1 Diabetes (Clinical Trials registration: NCT02218619), which aims to reduce endoplasmic reticulum stress and improve beta cell survival in patients with new onset Type 1 Diabetes. The lack of understanding about the molecular pathways activated by TUDCA hinders the development of new clinical studies.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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