PANCREAPEDIA
Exocrine Pancreas Knowledge Base

APA AMERICAN PANCREATIC ASSOCIATION

Pharmaceutical developments for chronic pancreatitis: pipelines and future options

Rajarshi Mukherjee and Robert Sutton

NIHR Liverpool Pancreas Biomedical Research Unit, Royal Liverpool and Broadgreen University Hospitals NHS Trust and Institute of Translational Medicine, University of Liverpool, Liverpool, UK.

e-mail: R.Sutton@liverpool.ac.uk

Version 1.0, July 28, 2016 [DOI: 10.3998/panc.2016.12]

1. Introduction

Chronic pancreatitis (CP) is a disease that remains without specific treatment and carries with it a substantial morbidity. The disease is a chronic inflammatory disease of the pancreas with the key hallmark being progressive fibrotic destruction of the pancreatic secretory parenchyma resulting in loss of acinar cells and islet cells and subsequent exocrine and endocrine insufficiency (13, 60). There is a significant variation in the epidemiology of CP amongst worldwide studies over the last forty years, mainly concentrated in the western world, indicating a range in incidence from 2.1 – 13.4 / 100,000 (46), with a twenty year mortality rate of 35.8 – 62% (47, 70). Numerous etiological factors have been identified: alcohol, nicotine, nutrition, hereditary / genetic, efferent duct / obstructive, autoimmune (60). Autoimmune pancreatitis, while recognized as a form of CP, is characterized by infiltration of lymphocytes and IgG4-positive plasma cells within the pancreatic parenchyma and responds significantly to steroid treatment, unlike other forms of CP, so will not be considered further in this review; nor will the management of pancreatic exocrine and/or endocrine insufficiency. While alcohol remains the most common etiological factor in most studies (16) only a small proportion of alcoholics develop chronic pancreatitis (70) suggesting a multi-factorial etiology to the disease. Our understanding of the interplay and contribution of risk factors has been greatly enhanced by genetic discovery, starting with the discovery nearly 20 years ago of mutations in the cationic trypsinogen gene (*PRSS1*) causing hereditary pancreatitis (107) to the recent identification of common genetic variants in *CLDN2* conferring an increased risk of alcohol-related CP, particularly in men (108).

The demand for novel treatments for CP has never been greater and this is based upon a number of factors. **[1]** The variation in epidemiology may be attributable to problems with long-term follow up, especially in chronic alcoholics, as well as common delays in obtaining a formal and standardised diagnosis. As a result, the disease burden is likely to be higher than previously reported (46). **[2]** No treatments are available to halt the progression of the disease and current treatment options for CP are limited to supportive and palliative care; patients with advanced disease can be managed with endoscopic and/or surgical pancreatic decompression, denervation, resection, bypass or transplantation (23, 95). **[3]** The patient impact of CP is significant both directly, with recurrent severe pain – the primary clinical complaint (3) – and repeated hospital admissions leading to a poor quality of life, as well as indirectly, through the complications of malnutrition and diabetes mellitus that result from exocrine and endocrine insufficiency. **[4]** The health resource burden as a

result of the disease is sizeable with estimated costs for both acute and chronic pancreatitis in the USA in 2004 amounting to $3.8 Billion (21). **[5]** A considerable number of patients presenting with acute pancreatitis (AP) may progress on to CP and risk factor control, be it from a hereditary etiology to a predominant alcoholic etiology, remains difficult. Population-based studies report that 20% – 45% of patients have a recurrence of AP, with the highest rates being amongst those with alcohol-related AP (71). Progression to CP after recurring AP has been reported in 4%–24% of patients, again more commonly amongst those with alcoholic recurrent AP (43). Interestingly, a long-term prospective study (1976 –1992) of patients who had recurring AP and continued to consume alcohol, disease progressed to CP in as many as 78% (2), with a 30-year Danish follow-up study finding that AP (alcohol-related and idiopathic) progressed to CP with a mean interval of 3.5 years (65). **[6]** CP carries a substantial risk of progression to pancreatic ductal adenocarcinoma (PDA). Patients with CP have a higher incidence of PDA (56), and individuals with hereditary pancreatitis have a 40% cumulative risk of developing PDA in their lifetime (99).

These crucial clinical characteristics of CP highlight the need for targeted novel treatment strategies to halt disease progression and thus improve patient outcomes. If novel drugs are combined with better standardised early diagnosis, a potentially significant impact on disease outcome may result. The identification of such putative treatment pipelines rests on a clear understanding of disease pathogenesis and mechanisms so that appropriate targets can be identified for drug discovery programmes as well as open options for drug repositioning.

2. Pathogenesis of CP and potential treatment strategies

The sentinel acute pancreatitis event (SAPE) hypothesis, first described by Whitcomb in 1999, provides a unified model for the pathogenesis of CP (105). After studying cases of hereditary pancreatitis, Whitcomb et al. found that 50% of patients with gain-of-function trypsinogen mutations experienced repeated episodes of AP that later developed into CP (86). Regardless of the cause of the sentinel event of AP, recurrent episodes of AP can progress to CP. CP is thus a complex multifactorial disease that requires the interaction of various environmental factors (e.g. alcohol consumption), recurrent injury (e.g. trypsin activation and autodigestion) and the immune response (106). AP is characterised by acinar and ductal cell injury, premature acinar zymogen activation, recruitment of inflammatory cells, auto-digestion and necrosis of acinar and ductal cells, subsequent reparative and anti-inflammatory responses, repetitive episodes of which drive pancreatic stellate cell (PSC) activation and PSC-dependent fibrosis (110). Recurrent and/or sustained pancreatic parenchymal injury and inflammation lead to progressive irreversible fibrosis (110), the pathological hallmark of CP. Pain, however, does not correlate well with morphological features of CP (109) and the extent to which primary parenchymal injury contributes to the progression of established CP is unclear. Nevertheless any strategy to modulate outcome in CP must be based on a detailed understanding of the pathological process of destruction of the pancreatic parenchyma and resultant fibrogenesis.

Our understanding of fibrogenesis in the pancreas of patients with CP improved with the finding that PSCs regulate synthesis and degradation of the extracellular matrix proteins (particularly fibronectin and fibrillary collagen types) that comprise fibrous tissue (67). Under normal homeostatic conditions, PSCs remain in their quiescent form but they can be activated by a variety of toxic factors, such as ethanol and its metabolites, or by inflammatory cytokines and chemokines, which are up-regulated in pancreatic tissues of patients with CP. Such factors induce PSCs to proliferate and transform into myofibroblast- like cells (6). Thus, novel therapeutic strategies could target one of three potential areas in the disease process: treatments

to reduce primary parenchymal injury, immunomodulation or pancreatic stellate cell inhibition **(Figure 1)**.

3. Immunology of CP

How immune factors contribute to disease pathogenesis and specifically PSC activation is an area of pivotal understanding that may produce numerous potential treatment pipelines. Immune cells play a key role in the pathogenesis of CP with a variety of changes observed in the condition **(Table 1)**. Infiltrating myeloid cells have previously been demonstrated to play a crucial role in PSC activation with activated macrophages previously shown to stimulate collagen and fibronectin synthesis by cultured PSCs (84), and furthermore by the requirement of myeloid (rather than acinar cell) nuclear factor-κB p65 subunit to promote fibrosis in experimental CP (94).

An increasing number of studies have focussed on the role of T cells in CP. An early study demonstrated pancreas samples to have significant increases in CD4+ and CD8+ T-cell infiltrates and *perforin* messenger RNA–expressing cells in CP lesions compared with healthy pancreatic tissue, indicating the likely involvement of cell-mediated cytotoxicity (35). Another study demonstrated no differences in total leukocyte or T-cell populations, however samples from patients with CP had increased numbers of CD4+ and CD8+ central memory T-cell subsets (CCR7+) compared with controls (28).

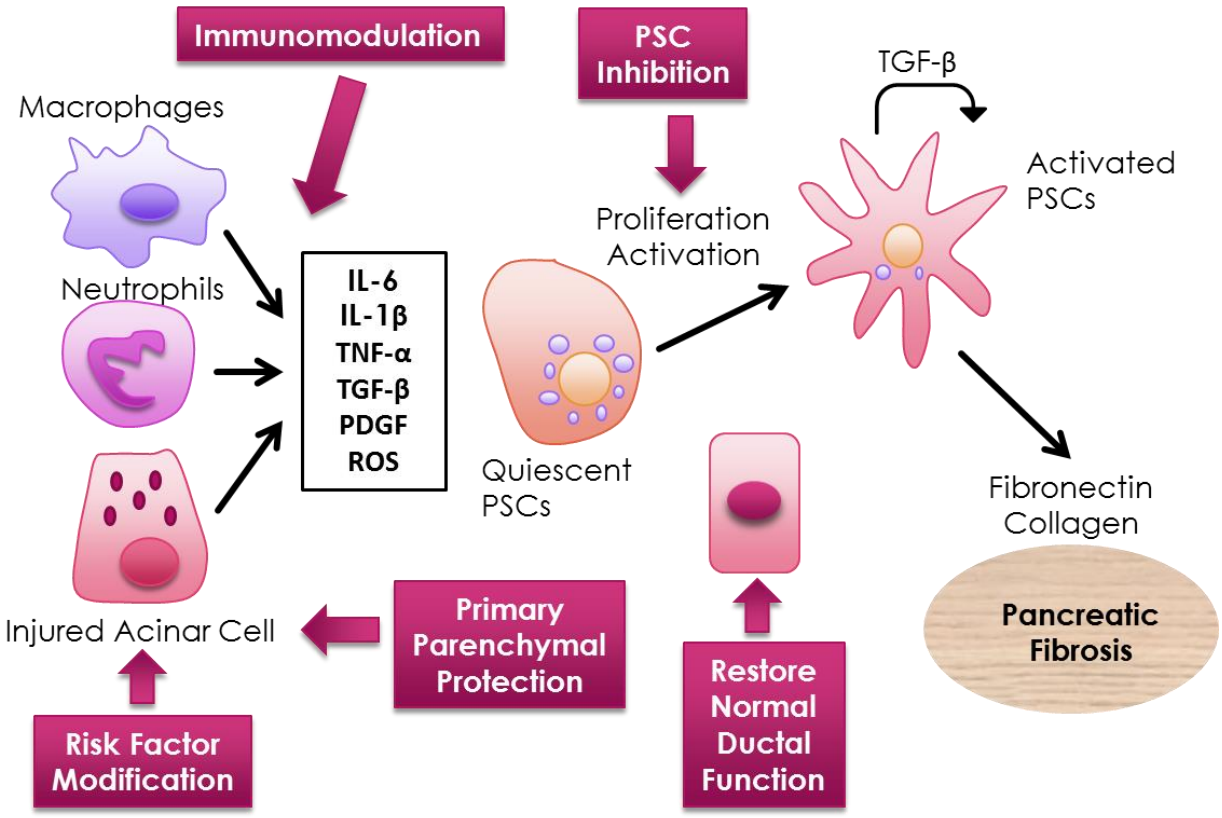

Figure 1. Potential therapeutic strategies for CP. The main areas that novel treatment strategies focus are risk factor modification, the restoration of normal ductal function in circumstances where this may be altered i.e. in CP with a predominant obstructive efferent duct aetiology, primary parenchymal protection, immunomodulation and pancreatic stellate cell (PSC) inhibition, applicable to all causes of CP. There exists a significant overlap between strategies targeting the immune system and pancreatic stellate cells with agents often affecting both.

Table 1: Summary of the key pathoimmunological responses observed in CP

Key immunological changes in CP	Reference
↑ **Myeloid cell pancreatic infiltrates, particularly macrophages**	Treiber et al., 2011 (94)
↑ **Inflam. cytokines (IL-6, IL-1β, TNF-α, TGF-β, PDGF, ROS)**	Mews et al., 2002 (57)
↑ **CD4+ and CD8+ T cell pancreas infiltrates**	Hunger et al., 1997 (35)
↑ **Circulating memory T cells**	Grundsten et al., 2005 (28)
Changes in memory and regulatory T cell responses	Schmitz-Winnenthal et al., 2010 (85)
↑ **Activation of PSCs**	Apte et al., 2005 (6)

Changes predominantly are observed in macrophage and T cell infiltrates, an increase in inflammatory cytokines, and increased activation of quiescent pancreatic stellate cells (PSC). Increasing evidence exists demonstrating changes in number and function of circulating memory and regulatory T cells.

A more recent study investigated pancreas-specific T cell responses to antigens from lysates of human CP lesions obtained during surgical resection (85).T cells from CP patients had higher levels of IL-10–based responses to pancreatitis-associated antigens compared to normal controls and patients with pancreatic ductal adenocarcinoma, supporting the association between CP and changes in tissue- and disease-specific memory and regulatory T-cell responses (85). The tragedy remains however that even in the light of these significant advances in our understanding of the pathoimmunology of CP there remains no immune-based therapies for the disease, but this could change in the future with significant recent advances in our understanding of the roles of PSCs and their interactions with immune and other pancreatic cells.

4. Pancreatic stellate cells: key to CP fibrosis

Amongst all pancreatic parenchymal cells, pancreatic stellate cells (PSCs) comprise 4–7% (7), and have been clearly established over the last twenty years as the key executors of pancreatic fibrogenesis. Indeed, numerous *in vitro* and *in vivo* studies clearly demonstrate the central role of activated PSCs in chronic pancreatitis associated fibrosis. PSCs are activated by a variety of toxic factors or by inflammatory cytokines and chemokines produced in CP, resulting in PSC proliferation and transformation into myofibroblast like cells (6) that produce the pancreatic fibrosis that characterises CP. The intracellular signalling mechanisms regulating PSC activation include the mitogen-activated protein kinase (MAPK) pathway, which plays a major role in ethanol- and acetaldehyde dependent activation of PSCs, phosphatidylinositol-3-kinase, and protein kinase C (54). The transition to the myofibroblast like phenotype is associated with increased expression of specific smooth muscle genes such as α smooth muscle actin (ACTA2) and transgelin (SM22α) and of specific markers such as cytoglobin/stellate cell activation associated protein (Cygb/STAP) in fibrotic lesions of the pancreas (62). Pancreatic stellate cells can be activated directly by alcohol consumption (5) or by cytokines derived from the immigrating inflammatory cells (33, 48). Platelet-derived growth factor is the major promoter of PSC migration, whereas transforming growth factor A (TGFA) affects ECM production via a Smad

associated pathway. Upon phosphorylation by the TGFA receptor, Smad3 enters the nucleus to modulate the transcription of target genes (79). Smad3 links TGFA signalling directly to the serum response factor (SRF)-associated regulatory network that controls the expression of smooth muscle-specific genes (74).

Although the earliest studies tended to primarily focus on the role of PSCs in pathological fibrosis, recently the maintenance of homeostasis within the pancreas by PSCs has been further explored (7), with roles in a number of physiological processes identified: the maintenance of normal ECM turnover; a role in cholecystokinin-mediated pancreatic exocrine secretion; recognition of pathogen-associated molecular patterns (PAMPs) via Toll-like receptors; a role in innate immunity by phagocytosing necrotic acinar cells and neutrophils; and the expression of stem cell markers with capacity to function as progenitor cells (4).

It is generally agreed that the PSCs in CP are mainly derived from the resident cells with some contribution from bone marrow derived pluripotent cells (36). Increasing evidence exists highlighting the role of PSCs in CP towards both exocrine and endocrine dysfunction. Increased PSC numbers have been detected in fibrotic areas around and within the islets of Langerhans in the pancreas of Goto-Kakizaki rats (a model of type 2 diabetes) and in-vitro work has shown that PSCs inhibit insulin secretion by beta cells as well as causing apoptosis of those cells. Recent studies have reported that hyperglycaemia aggravates the detrimental effects of PSCs on beta cell function (117), and that in hyperglycaemic mice, cerulein-induced chronic pancreatitis is significantly aggravated when compared with normoglycaemic mice (116).

Utilizing the understanding gained from these studies about the role of PSCs in chronic pancreatitis, many subsequent studies aimed at developing novel therapeutic approaches to minimize or reverse the fibrosis have been performed. These treatments have mostly been applied in established experimental models of pancreatic fibrosis frequently utilizing histopathological assessment and assays of PSC activation. Improvements in methods to isolate PSCs have allowed various previously difficult in vitro methods to be applied to the assessment of drug efficacy. A variety of therapeutic strategies have been tested with promising results in a range of experimental CP models over the last 10 years: antioxidants (119), inhibition of profibrogenic growth factors such as TGF-β (120), peroxisome proliferator-activated receptor gamma (PPARγ) ligands such as thiazolidinediones (38), protease inhibitors (25), a prostacyclin analogue ONO-1301 (64), the flavonoid apigenin and its analogues (58), inhibition of collagen synthesis by targeted treatment of PSCs with collagen siRNA (37), an anthraquinone derivative Rhein (96), amongst others **(Table 2)**.

The models of CP used have included repetitive caerulein injections over three to 10 weeks, the commonest model that has the advantage of targeting the pancreas; dibutyltin dichloride that induces fibrosis in the pancreas and liver; chronic ethanol administration with lipopolysaccharide, and combinations of these (45) as well as transgenic animals e.g. those expressing normal and mutated human cationic trypsinogen genes (9). The above studies are encouraging as potential treatments for pancreatic fibrosis in CP but the real challenge lies in translating these preclinical findings to the clinical setting. Amongst these studies, a variety of techniques ranging from *in vitro* to *in vitro* and *in vivo* using both mouse and human tissue, have been employed and some of the more promising treatments are appraised in more detail in the subsequent sections. Nevertheless greater standardisation is required in both preclinical models and clinical trial designs, the latter being especially underdeveloped for drug trials.

Table 2: Summary of key molecular targets and putative treatments tested in experimental CP in the last 10 years

Target / Drug	Model of CP	Findings	*Reference*
TGF-β / Adenoviral Vector expressing AdTb-ExR / Halofuginone	C57BL/6 mice Cerulein for 3 wks C57BL/6 mice Cerulein for 4/8 wks	Reduced fibrosis and reduced activated PSCs Reduced fibrosis	*Nagashio et al., 2004 (61)* *Zion et al., 2009 (120)*
Protease Inhibitors / Camostat mesilate	DBTC Rat for 4 wks with treatment at 1wk Cultured PSCs	Reduced fibrosis and PSC activation	*Gibo et al., 2005 (25)*
PPAR-γ /Thiazolidinediones	Immortalised Rat PSCs	Reduced PSC activation	*Jaster et al., 2005 (38)*
Mucolytic/Bromhexine Hydrochloride	12 human patients	Improvement in pain and exocrine function	*Tsujimoto et al., 2005 (97)*
Curcumin	Cultured Rat PSCs	Reduced activation and proliferation	*Masamune et al., 2006 (50)*
Green tea	Isolated cultured rat PSCs	Inhibited PSC activation	*Asaumi et al., 2006 (8)*
COX-2 / Rofecoxib	WBN/Kob Rats	Reduction in macrophage infiltration and fibrosis	*Reding et al., 2006 (76)*
MPTP / Tocotrienol (Vit. E derivative)	Isolated Rat PSCs	Induce Activated PSC death	*Rickmann et al., 2007 (77)*
Interferon-γ	Isolated Rat PSCs	Reduce PSC activation	*Fitzner et al., 2007 (22)*
Withdrawal of alcohol	Rats fed alcohol diet for 10 wks then LPS for 3 wks	Improvement in fibrosis and decreased PSC apoptosis	*Vonlaufen et al., 2010 (100)*
Rapamycin	DBTC & Cerulein Rats	Reduced fibrosis, preservation of normoglycaemia	*Mayer et al, 2012 (53)*
Collagen siRNA to PSC / VA-lip-siRNAgp46	DBTC & Cerulein Rats,	Resolution of pancreatic fibrosis	*Ishiwatari et al., 2013 (37)*
Rhein (anthraquinone deriv.)	C57BL/6 mice Cerulein for 6 wks, Treatment given on induction and later at 4wks	Decreased PSC activation and fibrosis in both intervention groups	*Tsang et al., 2013 (96)*
ROS / Edaravone	DBTC Rat for 4 weeks; treatment after 2 weeks	Reduced fibrosis, PSC activation and cytokine expression	*Zhou et al., 2013 (119)*
ONO-1301 (Prostacyclin analogue)	DBTC rats, Treatment initiation at 1 wk, Sacrifice 2 & 3 wks	Decrease in inflam. Infiltrate 2 wks & fibrosis 3 wks	*Niina et al., 2014 (64)*
Apigenin (Flavonoid)	C57BL/6 mice Cerulein Treatment initiation at 1 wk, Sacrifice at 4 ks	Decreased fibrosis and PSC activation	*Mrazick et al., 2015 (58)*
IL-4/IL-13	C57BL/6 Cerulein, IL-4/IL-13 -/- mice, Human tissue	Inhibition decreases alternatively activated macrophages and fibrosis	*Xue et al., 2015 (112)*

Most have employed standard cerulein mouse models of CP with assays of pancreatic fibrosis and PSC activation most commonly used as endpoints to assess efficacy (studies in chronological order; DBTC = dibutyltin dichloride; MPTP = mitochondrial permeability transition pore; ROS = reactive oxygen species).

5. Primary parenchymal protection as a treatment strategy

The repetitive and/or continuous injury of the pancreatic parenchyma inflicted by toxic, metabolic, genetic and other causes first and foremost damages the cells making up the vast majority of the parenchyma – the acinar cells – as well as the ductal cells (20). Both cell types are injured by fatty acid ethyl esters, non-oxidative metabolites of ethanol, and fatty acids that are implicated in alcohol-associated and hyperlipidaemic AP and CP (17, 18, 34, 49). Both induce cytosolic calcium overload that in turn induces mitochondrial calcium overload, compromising the supply of ATP and inhibiting autophagy that would otherwise clear the associated premature intracellular digestive enzyme activation. The compromise in ATP production occurs through excessive mitochondrial matrix calcium concentrations that induce the mitochondrial permeability transition pore, likely formed by the F_0F_1ATP synthase and regulated by cyclophilin D, allowing molecules <1500 Daltons to pass through the inner mitochondrial membrane (59). Mitochondrial membrane potential is lost, ATP production compromised and cellular necrosis results, inducing the necro-inflammatory sequences that drive AP and, likely with repetitive injury, CP. Similar events occur in hyperstimulation-induced AP and CP, exploited in the repetitive caerulein injection model of CP, the most widely used model **(Table 2)**. The severity of both experimental AP and CP is dependent on the dose of the toxin and the number of times repeated. Treatments that either inhibit calcium entry into pancreatic parenchymal cells or protect mitochondria have been shown to be highly effective in experimental AP (59, 102), and could have a place in the treatment of CP. Thus inhibition of the principal store-operated calcium channel Orai1 has been shown to markedly reduce the severity of experimental AP and inhibition of cyclophilin D has almost removed all pathological consequences in some models of experimental AP. The latter strategy is especially attractive as cyclophilin D knockout is compatible with viability in utero and only a modest murine phenotype, whereas constitutive Orai1 knockout is not viable in utero. There is evidence that primary parenchymal protection is a workable strategy from studies of rapamycin in rats administered dibutyltin dichloride and cerulein to induce CP (53), which acts at least in part to protect the mitochondrial compartment (27, 72). Nevertheless the approach requires further preclinical validation and the development of agents that are safe and can be administered orally over prolonged periods, if not indefinitely.

6. Cytokine inhibition

Cytokines as signalling molecules play a major role in the pathogenesis of CP and while they may be a disparate group with many individual cytokines and are often pleiotropic, they remain key factors for cell-cell signalling and PSC activation and thus important potential targets for CP. Indeed, numerous strategies have been employed over the years to target cytokine signalling and attempt to develop treatments that might improve outcomes in CP.

Transforming growth factor-β (TGF-β) is thought to regulate the production, degradation, and accumulation of extracellular matrix (ECM) proteins, and to play an important role in the fibro proliferative changes that follow tissue injury in many vital organs and tissues, including the heart, lung, kidney, and liver (14, 51). The importance of TGF-β signalling in the formation of fibrosis is underlined by experiments in transgenic mice overexpressing TGF-β1 in the pancreas (44, 80). These animals show histological changes that resemble human chronic pancreatitis including destruction of the exocrine pancreas and progressive accumulation of ECM in the pancreas. Pharmacological TGF-β inhibition holds promise as a treatment strategy. Halofuginone, an analogue of the plant alkaloid febrifugine, was recently tested in a cerulein experimental CP mouse model (120). Halofuginone was found to prevent cerulein-dependent increase in collagen

synthesis, collagen cross-linking enzyme P4HA, Cygb/STAP, and tissue inhibitors of metalloproteinase 2, through inhibition of serum response factor and the downstream TGF-β signalling component, Smad3 phosphorylation. Furthermore, *in vitro* cultured pancreatic stellate cell (PSC) proliferation and TGF-β dependent increase in Cygb/STAP and transgelin synthesis and metalloproteinase 2 activity was inhibited. Few specific TGF-β receptor kinase inhibitors exist however and while compounds such as SB-431542 that are being developed for the treatment of neoplasia (30), are available, potential applications in CP of these inhibitors remain to be explored. Gene therapy has been assessed to specifically target TGF-β (61) and shall be discussed further in the next section.

Interferons (IFNs) are multifunctional cytokines that block viral infection, modulate immune as well as inflammatory responses, and inhibit cell proliferation (92). IFN-α is an effective drug already established in clinical practice for the treatment of patients with chronic hepatitis B or C associated with liver fibrosis (63, 87), acting partly through an inhibitory effect on hepatic stellate cells (12, 89). However, conflicting evidence exists about their potential role in CP. IFN-γ, but not IFN-α has been demonstrated to display inhibitory effects on PSC proliferation and collagen synthesis *in vitro* using recombinant rat IFN on isolated rat PSCs, but IFN-γ has been shown to decrease glucose stimulated insulin release from islet cells and thus potentially play a role in CP endocrine dysfunction (69). IFN-α in combination with ribavirin has been associated with drug-induced acute pancreatitis (19), so although IFNs may still be of potential use as novel treatments in the chronic form of the disease, further characterisation of their molecular effects is required before proceeding with further drug development. Similarly, TNF-α and IL-6 have both been demonstrated to be upregulated in CP and be involved in immune cell signalling as well as activation of quiescent PSCs (6) but modulating strategies using experimental and clinical anti-TNF (infliximab, golimumab) or anti-IL-6 (tocilizumab) agents (82) are yet to be explored in CP. The clinical use of licensed biologics, however, has increased in many inflammatory and other diseases over the last two decades such that this type of drug accounts for a major share of all drugs administered. Repositioning of a licensed drug or biological response modifier has many attractions, not least that the expense of drug development is substantially reduced.

Recent evidence suggests that pharmacological inhibition of interleukin-4 (IL-4) and interleukin-13 (IL-13) may hold significant potential in the treatment of CP. A very detailed and wide-ranging study was undertaken utilising *in vitro*, *in vivo* and *ex vivo* approaches, assessing both transgenic mouse models and human pancreatic tissue from CP patients, focussing on the interaction between alternatively activated macrophages (AAMs) and PSCs through IL-4/IL-13 signalling (112). The investigators found that AAMs are dominant in mouse and human CP and that they are dependent on interleukin IL-4 and IL-13 signalling. Furthermore they observed that mice lacking IL-4Ra, myeloid-specific IL-4Ra and IL-4/IL-13 were less susceptible to pancreatic fibrosis, with mouse and human PSCs being a source of IL-4/IL-13. Finally, and probably most importantly, they showed that pharmacologic inhibition of IL-4/IL-13 using IL-4/IL-13 blocking peptide administered half way through the course of an established mouse CP model as well as in human ex vivo studies, decreased pancreatic AAMs and fibrosis (112). Thus, as one of the most thorough studies published in the CP literature to date, the strategy of IL-4/IL-13 inhibition does hold promise as a novel treatment pipeline for CP and identifies other potential immune targets associated with AAMs that may also be considered for targeting. As an example of possibilities with this target, Regeneron has developed dupilumab, an inhibitor of IL-4Rα, which is at an advanced stage of development for atopic disease (103). There are thus significant possibilities for targeting cytokines in the treatment of CP (111), yet to be explored in a major way both experimentally and clinically.

7. Treatments based on natural compounds

Natural products have in the past been a rich source of compounds for drug discovery, but their use has somewhat diminished, partly due to the technical barriers to screening natural products in high-throughput assays against molecular targets (31). Recent strategies have often employed natural product screening that utilize recent technical advances in genomic and metabolomics approaches to augment traditional methods of studying natural products with an appreciation of functional assays and phenotypic screens specific to the particular disease under consideration with the most applications till date in the fields of cancer and microbiology (10, 39). The use of natural products as a base to guide drug discovery for CP has been increasingly implemented over the last ten years (5, 92, 120) with a number of compounds showing promise in experimental CP models. Polyphenols, extracted from green tea, have been found to have inhibitory effects on isolated rat PSC activation and may be able to prevent the pancreatic fibrosis of CP (8). Likewise, Curcumin (diferuloyl-methane), a natural product from the spice turmeric (26) has a variety of biological activities including anti-inflammatory (29, 93), antioxidant (75), antifibrotic (40, 73), and has previously been shown to inhibit activation of isolated PSCs *in vitro* (50). Vitamin A (retinol) and its metabolites all-trans retinoic acid (ATRA) and 9-cis retinoic acid (9-RA) were found to significantly inhibit proliferation and activation of cultured PSCs (55). While further studies to evaluate these compounds in *in vivo* conditions are awaited a number of natural compounds have been explored in more detail in the setting of CP.

Apigenin (4´,5,7-trihydroxyflavone) is a natural compound with low intrinsic toxicity, found in various fruits, vegetables, herbs, and beverages such as chamomile tea (90). A recent study reported apigenin treatment in a standard cerulein model of experimental CP, inhibited PSC proliferation, induced PSC apoptosis and minimized parathyroid hormone related peptide (PTHrP)-mediated PSC response to injury (58). Furthermore novel analogues of Apigenin are under development with chemical modifications directed to build a focused library of *O*-alkylamino-tethered apigenin derivatives at 4'-*O* position of the ring C with the aim of enhancing the potency and overall drug-like properties including aqueous solubility (15).

Rhein is a natural anthraquinone derivative, also known chemically as 9,10-Dihydro-4, 5-dihydroxy-9, 10-dioxo-2-anthracenecarboxylic acid, that can be extracted from roots of Polygonaceae (rhubarb) (96). This yellow crystalline rhubarb extract has been serving as a mild laxative agent as well as an astringent since ancient times in the Chinese population (113). In recent decades, administration of rhein in the range of 25 to 100 mg/kg/day has been demonstrated to exert diverse pharmacological actions including anti-microbial (101), anti-angiogenic (32) and anti-cancer activities (114). Rhein when administered at 50 mg/kg/day half way through the course of an experimental cerulein CP mouse model was able to reverse fibrotic outcomes and when administered in vitro was found to attenuate PSC activation and suppress sonic hedgehog (SHH) signalling (96).

Recent evidence suggests that the mitochondrial permeability transition pore (MPTP), a gatekeeper for cell death pathways in the injured cell, may be a crucial target for drug discovery in AP (59), however as indicated previously (section 5) its potential use in CP is yet to be fully explored. Tocotrienol (α, β, γ, δ) along with tocopherol (α, β, γ, δ) stereoisomers represent the two naturally occurring subclasses of vitamin E compounds. Although the diet of millions of people includes tocotrienol- rich foods such as palm oil or rice bran, more than 95% of the scientific literature on vitamin E has focused exclusively on α-tocopherol (88). Despite some previous concerns on their bioavailability, it is now clear that dietary tocotrienols are well absorbed, show measurable plasma levels (42) and are readily distributed

throughout the tissues (68). Accumulating evidence suggests that tocotrienols display greater beneficial effects than α-tocopherol because of their prominent antineoplastic, neuroprotective, cardioprotective, and cholesterol-lowering properties (88). A recent study using a tocotrienol rich fraction (TRF) from palm oil found that TRF, but not α-tocopherol, reduced viability of activated PSCs (not quiescent PSCs or isolated acinar cells) *in vitro* through apoptosis and autophagy and caused a sustained mitochondrial membrane depolarization and extensive cytochrome c release that was completely abolished with the MPTP inhibitor cyclosporine A (77).

Although the findings from drugs developed based on natural compounds on isolated PSCs show promise (77), one must remember that these findings along with many others using natural compounds on only isolated cells, require validating in experimental CP models as well as ultimately human CP. The main challenge remains in refining compounds with regards to specificity for cell type and specificity of action, and this should remain the main focus of ongoing research.

8. Gene therapy strategies

Gene therapy strategies provide a distinct advantage in terms of treatment specificity and have been utilised in various CP studies. While pharmacological inhibition of TGF-β inhibition has previously been considered, inhibition employing an adenoviral vector expressing the entire extracellular domain of type II human TGF-β receptor (AdTβ-ExR) on a cerulein mouse model of experimental CP has also been tested (61). The study evaluated pancreatic fibrosis, PSC activation, apoptosis and proliferation of acinar cells, by histology and immunostaining and found in AdTβ-ExR-injected mice, pancreatic fibrosis was significantly attenuated with a reduction of activated PSCs and apoptotic acinar cells but no change on proliferation (61). Targeted encephalin gene therapy has been shown to reduce pain in experimental CP (104), but is unlikely to modify disease progression. Further research indicates that gene therapy may hold potential promise specifically in CP patients carrying a CFTR mutation (11). Use of this strategy in other chronic inflammatory diseases (such as primary Sjögren syndrome), using exogenous gene delivery of aquaporin water channels into the parotid glands of patients, has been successfully applied to treat the dry mouth symptoms that form part of the condition (115). As an aside, the changes of pancreatic ductal fluid and ion concentration in pancreatitis are very similar to the mechanisms visible in cystic fibrosis (CF) (11). Therefore, drugs which are effective in CF may can have benefits for patients suffering with CP, such as bromhexine hydrochloride, a bronchial mucolytic, that when administered to 12 patients with alcoholic CP showed improvements in symptoms and exocrine function (97).

Clearly like many other conditions, while having the advantage of being specific in nature, adopting gene therapy as an approach in CP remains challenging. This strategy is open to various potential drawbacks that have been discussed in length in the recent literature: CP is a multifactorial disorder with a polygenic predisposition; long term outcomes remain unclear posing a number of ethical issues; risks may exist from induction of tumour growth; initiation of the endogenous immune response and the use of viral vectors for gene transmission may carry risk (41).

A strategy that harnesses the benefits of specific genetic technologies and bypasses the problems that may be associated with viral adenovectors is the use of small interfering RNA (siRNA) to target a relevant mRNA, key to the pathogenesis of CP, for degradation. Previous studies have demonstrated that siRNA against collagen-specific chaperone protein gp46, encapsulated in vitamin A-coupled liposomes (VA-lip-siRNAgp46), resolved fibrosis in a model of liver cirrhosis (81). Subsequently the treatment was assessed as a treatment for pancreatic fibrosis in experimental dibutyltin dichloride (DBTC) and cerulein induced CP in rats (37). The experimenters were able to demonstrate specific uptake of VA-lipsiRNAgp46

by conjugation with 60-carboxyfluorescein (FAM) followed by immunofluorescence showing uptake through the retinol binding protein receptor by activated PSCs *in vitro*, accompanied by successful knockdown of gp46 and suppression of collagen secretion. The technique allowed specific delivery of VA-lip-siRNAgp46 to PSCs in fibrotic areas in DBTC rats with 10 systemic treatments resolving pancreatic fibrosis, and suppressing tissue hydroxyproline levels in both models (37). While full translation of such siRNA strategies to the clinical setting remains some distance away, this study provides the first key demonstration of successful targeting of an antifibrotic drug to cells known to be responsible for pancreatic fibrosis and creates hope that similar strategies may be employed, potentially with other similar or even contrasting drug targets, to alter the course of CP.

9. Other approaches

A number of other drugs and strategies have been recently explored as treatments for CP with some promising findings. Camostat mesilate (CM), an oral protease inhibitor, has been used clinically for the treatment of chronic pancreatitis in Japan (25). This is mainly based on the theoretical benefit of decreasing prematurely activated trypsinogen in the pancreas, that is a key feature of acute acinar cell injury from a variety of pancreatic toxins (78). Interestingly, CM has been shown to attenuate DBTC-induced rat pancreatic fibrosis probably via inhibition of monocytes and PSC activity (25). However, a recent study employing transgenic mice conditionally expressing an endogenously activated trypsinogen within pancreatic acinar cells demonstrated that trypsin-mediated injury was sufficient for AP, but in the absence of other factors was not sufficient to drive pancreatic fibrosis and CP, raising questions as to the utility of protease inhibition as a strategy in CP (24).

Cyclooxygenase (COX) is an enzyme that produces prostaglandins, such as prostacyclin and thromboxane, with COX-2 being unexpressed under normal conditions in most cells but elevated during inflammation. Modulation of prostaglandins in CP has produced some conflicting findings. Numerous chronic inflammatory diseases can be successfully suppressed by COX-2 inhibitors (52) and COX-2 is elevated in CP (83). A recent study assessed administration of the selective COX-2 inhibitor, rofecoxib, on an experimental model of CP (WBN/Kob rat) with a reduction in chronic inflammatory changes and fibrosis following treatment with in vitro studies suggesting migration of macrophages in CP conditions to be COX-2 dependent (76). This would suggest a beneficial effect from the reduction of prostaglandins, including prostacyclin, for CP, in line with other inflammatory conditions. However understanding this treatment strategy remains complex as a further recent study using ONO-1301, a novel sustained-release prostacyclin analogue shown to have anti-fibrotic effects in other organs, resulted in an improvement in fibrosis in a dibutylin chloride (DBTC) rat model of CP although *in vitro* studies showed no effect of ONO-1301 on PSCs (64). Clearly, COX-2 inhibition will lead to a decrease of prostaglandins other than prostacyclin, such as thromboxane, and this may be responsible for an overriding beneficial effect observed by this treatment strategy. Overall, these studies highlight that further characterisation of this mechanistic pathway in the setting of CP is required to guide better drug development.

Braganza first proposed that CP arose as a result of oxidative stress and that a deficient free radical quenching system combined with excess free radical production led to cellular injury (98). Reactive oxygen species are known to be involved in PSC activation (4) and theoretically play an important role in pathogenesis of CP. Braganza et al. (98) reasoned that exogenous supplementation with antioxidants or precursors for antioxidant pathways might help to reduce ongoing acinar injury. After a small randomized trial of selenium, β-carotene, vitamins C and E, and methionine-based antioxidant therapy reported a reduction in severity and frequency of episodes of pain in patients with recurrent and chronic pancreatitis, a commercially available formulation was developed, however, antioxidant therapy for chronic

pancreatitis has not become accepted as standard therapy, with recent trials suggesting administration of antioxidants to patients with CP does not improve quality of life (91) and a recent Cochrane review suggesting they may have only a small beneficial effect on pain (1).

Many lessons can be learnt from the antioxidant treatment pipeline that can be implemented for other future strategies that may involve targets and compounds previously outlined in this review: the timing of intervention in the pathological process of fibrogenesis remains crucial and studies allowing cross-comparability of interventional time points in preclinical studies with human CP are further required; trials must use standardised clearly defined criteria for diagnosis of CP and hence the inclusion of the most appropriate patients in trials; the composition of test compounds must be refined and standardised with multiple constituent strategies causing inevitable difficulties in cross-comparison between studies; relevant disease outcome measures must be standardised and caution must be exercised in interpretation of subjective measures, such as pain and quality of life scores, alongside objective measures such as endocrine and exocrine insufficiency.

10. Conclusion

Multiple novel treatment pipelines have been identified by preclinical studies in CP over the last decade **(Figure 2)**, with recent investigation focussed on parenchymal protection, immunomodulation and PSC inhibition as strategies to reduce pancreatic injury and fibrosis (118) and reduce the symptomatic and long-term impacts of the disease. Ultimately, whether these promising preclinical findings can have an impact on human CP will depend on translation through well-structured and co-ordinated clinical trials. Trials, to date, have not provided any disease-course altering specific treatments, with many promising compounds still to be tested. There remains many pharmacological challenges in human CP however, that must be overcome for effective translation of preclinical findings. Drug absorption in patients with chronic pancreatitis might be affected by the pathophysiology of the disease, with exocrine insufficiency associated with changes in gastrointestinal intraluminal pH, motility disorder, bacterial overgrowth and changed pancreatic gland secretion, resulting in potential malabsorption (66). Coupled with this, the lifestyle of CP patients may also contribute to these pharmacological challenges with many patients limiting their food intake due to pain caused by eating that will affect drug absorption and compliance, as well as alcohol and drug interactions known to influence pharmacokinetics (66). Nevertheless, much hope still exists that future research will provide successful treatments. These treatments will likely originate from pre-identified or novel drug targets based on a thorough understanding of pathogenesis, accompanied by clever drug design sensitive to the challenging group of CP patients, supported by sufficiently large and well-conducted clinical trials, with focussed research to translate from bench to bedside.

Acknowledgement

We acknowledge funding support from CORE, the UK Medical Research Council and the Biomedical Research Unit Funding scheme of the UK National Institute for Health Research. Robert Sutton is an NIHR Senior Investigator.

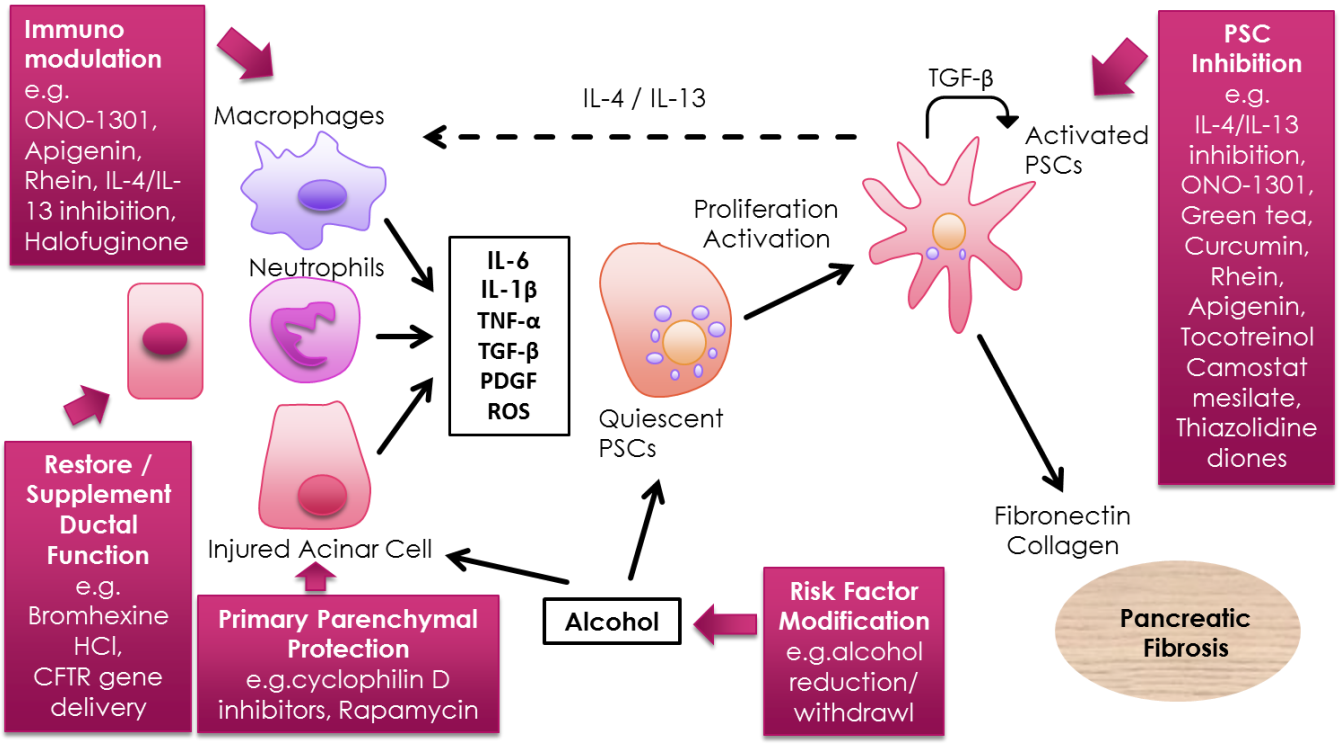

Figure 2. Summary of novel treatment pipelines for CP. Numerous agents tested in the pre-clinical setting have been shown to be efficacious in improving experimental CP outcomes (predominantly PSC activation and histopathological evidence of pancreatic fibrosis) with many agents chiefly acting through modulation of either immune pathways, PSC activation or both. Alcohol may act indirectly through repeated acinar cell injury or directly on PSCs to exert its deleterious effects. Recent evidence suggests an amplifying loop exists between alternatively activated macrophages and PSCs in CP through IL-4/IL-13 signalling, offering another therapeutic target.

11. References

1. **Ahmed Ali U, Jens S, Busch OR, Keus F, van Goor H, Gooszen HG, et al.** Antioxidants for pain in chronic pancreatitis. *Cochrane Database Syst Rev* 8: CD008945, 2014. PMID: 25144441.
2. **Ammann RW, Muellhaupt B, Meyenberger C and Heitz PU**. Alcoholic nonprogressive chronic pancreatitis: prospective long-term study of a large cohort with alcoholic acute pancreatitis (1976-1992). *Pancreas* 9(3): 365-373, 1994. PMID: 8022760.
3. **Anderson MA, Akshintala V, Albers KM, Amann ST, Belfer I, Brand R, et al.** Mechanism, assessment and management of pain in chronic pancreatitis: Recommendations of a multidisciplinary study group. *Pancreatology* 16(1): 83-94, 2016. PMID: 26620965.
4. **Apte M, Pirola RC and Wilson JS**. Pancreatic stellate cell: physiologic role, role in fibrosis and cancer. *Curr Opin Gastroenterol* 31(5): 416-423, 2015. PMID: 26125317.
5. **Apte MV, Phillips PA, Fahmy RG, Darby SJ, Rodgers SC, McCaughan GW, et al.** Does alcohol directly stimulate pancreatic fibrogenesis? Studies with rat pancreatic stellate cells. *Gastroenterology* 118(4): 780-794, 2000. PMID: 10734030.
6. **Apte MV, Pirola RC and Wilson JS**. Molecular mechanisms of alcoholic pancreatitis. *Dig Dis* 23(3-4): 232-240, 2005. PMID: 16508287.
7. **Apte MV, Pirola RC and Wilson JS**. Pancreatic stellate cells: a starring role in normal and diseased pancreas. *Front Physiol* 3: 344, 2012. PMID: 22973234.
8. **Asaumi H, Watanabe S, Taguchi M, Tashiro M, Nagashio Y, Nomiyama Y, et al.** Green tea polyphenol (-)-epigallocatechin-3-gallate inhibits ethanol-induced activation of pancreatic stellate cells. *Eur J Clin Invest* 36(2): 113-122, 2006. PMID: 16436093.
9. **Athwal T, Huang W, Mukherjee R, Latawiec D, Chvanov M, Clarke R, et al.** Expression of human cationic trypsinogen (PRSS1) in murine acinar cells promotes pancreatitis and apoptotic cell death. *Cell Death Dis* 5: e1165, 2014. PMID: 24722290.

10. **Balakrishnan K and Gandhi V**. Bcl-2 antagonists: a proof of concept for CLL therapy. *Invest New Drugs* 31(5): 1384-1394, 2013. PMID: 23907405.
11. **Balazs A and Hegyi P**. Cystic fibrosis-style changes in the early phase of pancreatitis. *Clin Res Hepatol Gastroenterol* 39 Suppl 1: S12-17, 2015. PMID: 26206571.
12. **Baroni GS, D'Ambrosio L, Curto P, Casini A, Mancini R, Jezequel AM, et al.** Interferon gamma decreases hepatic stellate cell activation and extracellular matrix deposition in rat liver fibrosis. *Hepatology* 23(5): 1189-1199, 1996. PMID: 8621153.
13. **Brock C, Nielsen LM, Lelic D and Drewes AM**. Pathophysiology of chronic pancreatitis. *World J Gastroenterol* 19(42): 7231-7240, 2013. PMID: 24259953.
14. **Broekelmann TJ, Limper AH, Colby TV and McDonald JA**. Transforming growth factor beta 1 is present at sites of extracellular matrix gene expression in human pulmonary fibrosis. *Proc Natl Acad Sci U S A* 88(15): 6642-6646, 1991. PMID: 1862087.
15. **Chen H, Mrazek AA, Wang X, Ding C, Ding Y, Porro LJ, et al.** Design, synthesis, and characterization of novel apigenin analogues that suppress pancreatic stellate cell proliferation in vitro and associated pancreatic fibrosis in vivo. *Bioorg Med Chem* 22(13): 3393-3404, 2014. PMID: 24837156.
16. **Cote GA, Yadav D, Slivka A, Hawes RH, Anderson MA, Burton FR, et al.** Alcohol and smoking as risk factors in an epidemiology study of patients with chronic pancreatitis. *Clin Gastroenterol Hepatol* 9(3): 266-273; quiz e227, 2011. PMID: 21029787.
17. **Criddle DN, Murphy J, Fistetto G, Barrow S, Tepikin AV, Neoptolemos JP, et al.** Fatty acid ethyl esters cause pancreatic calcium toxicity via inositol trisphosphate receptors and loss of ATP synthesis. *Gastroenterology* 130(3): 781-793, 2006. PMID: 16530519.
18. **Criddle DN, Raraty MG, Neoptolemos JP, Tepikin AV, Petersen OH and Sutton R**. Ethanol toxicity in pancreatic acinar cells: mediation by nonoxidative fatty acid metabolites. *Proc Natl Acad Sci U S A* 101(29): 10738-10743, 2004. PMID: 15247419.
19. **Eland IA, Rasch MC, Sturkenboom MJ, Bekkering FC, Brouwer JT, Delwaide J, et al.** Acute pancreatitis attributed to the use of interferon alfa-2b. *Gastroenterology* 119(1): 230-233, 2000. PMID: 10889173.
20. **Etemad B and Whitcomb DC**. Chronic pancreatitis: diagnosis, classification, and new genetic developments. *Gastroenterology* 120(3): 682-707, 2001. PMID: 11179244.
21. **Everhart JE and Ruhl CE**. Burden of digestive diseases in the United States Part III: Liver, biliary tract, and pancreas. *Gastroenterology* 136(4): 1134-1144, 2009. PMID: 19245868.
22. **Fitzner B, Brock P, Nechutova H, Glass A, Karopka T, Koczan D, et al.** Inhibitory effects of interferon-gamma on activation of rat pancreatic stellate cells are mediated by STAT1 and involve down-regulation of CTGF expression. *Cell Signal* 19(4): 782-790, 2007. PMID: 17116388.
23. **Forsmark CE**. Management of chronic pancreatitis. *Gastroenterology* 144(6): 1282-1291 e1283, 2013. PMID: 23622138.
24. **Gaiser S, Daniluk J, Liu Y, Tsou L, Chu J, Lee W, et al.** Intracellular activation of trypsinogen in transgenic mice induces acute but not chronic pancreatitis. *Gut* 60(10): 1379-1388, 2011. PMID: 21471572.
25. **Gibo J, Ito T, Kawabe K, Hisano T, Inoue M, Fujimori N, et al.** Camostat mesilate attenuates pancreatic fibrosis via inhibition of monocytes and pancreatic stellate cells activity. *Lab Invest* 85(1): 75-89, 2005. PMID: 15531908.
26. **Govindarajan VS**. Turmeric--chemistry, technology, and quality. *Crit Rev Food Sci Nutr* 12(3): 199-301, 1980. PMID: 6993103.
27. **Green DR, Galluzzi L and Kroemer G**. Cell biology. Metabolic control of cell death. *Science* 345(6203): 1250256, 2014. PMID: 25237106.
28. **Grundsten M, Liu GZ, Permert J, Hjelmstrom P and Tsai JA**. Increased central memory T cells in patients with chronic pancreatitis. *Pancreatology* 5(2-3): 177-182, 2005. PMID: 15849488.
29. **Gukovsky I, Reyes CN, Vaquero EC, Gukovskaya AS and Pandol SJ**. Curcumin ameliorates ethanol and nonethanol experimental pancreatitis. *Am J Physiol Gastrointest Liver Physiol* 284(1): G85-95, 2003. PMID: 12488237.
30. **Halder SK, Beauchamp RD and Datta PK**. A specific inhibitor of TGF-beta receptor kinase, SB-431542, as a potent antitumor agent for human cancers. *Neoplasia* 7(5): 509-521, 2005. PMID: 15967103.
31. **Harvey AL, Edrada-Ebel R and Quinn RJ**. The re-emergence of natural products for drug discovery in the genomics era. *Nat Rev Drug Discov* 14(2): 111-129, 2015. PMID: 25614221.
32. **He ZH, Zhou R, He MF, Lau CB, Yue GG, Ge W, et al.** Anti-angiogenic effect and mechanism of rhein from Rhizoma Rhei. *Phytomedicine* 18(6): 470-478, 2011. PMID: 21112197.
33. **Hirose H, Maruyama H, Kido K, Ito K, Koyama K, Tashiro Y, et al.** Defective insulin and glucagon secretion in isolated perfused pancreata of diabetic WBN/Kob rats. *Pancreas* 10(1): 71-77, 1995. PMID: 7899463.

34. **Huang W, Booth DM, Cane MC, Chvanov M, Javed MA, Elliott VL, et al.** Fatty acid ethyl ester synthase inhibition ameliorates ethanol-induced Ca2+-dependent mitochondrial dysfunction and acute pancreatitis. *Gut* 63(8): 1313-1324, 2014. PMID: 24162590.
35. **Hunger RE, Mueller C, Z'Graggen K, Friess H and Buchler MW**. Cytotoxic cells are activated in cellular infiltrates of alcoholic chronic pancreatitis. *Gastroenterology* 112(5): 1656-1663, 1997. PMID: 9136845.
36. **Ino K, Masuya M, Tawara I, Miyata E, Oda K, Nakamori Y, et al.** Monocytes infiltrate the pancreas via the MCP-1/CCR2 pathway and differentiate into stellate cells. *PLoS One* 9(1): e84889, 2014. PMID: 24416305.
37. **Ishiwatari H, Sato Y, Murase K, Yoneda A, Fujita R, Nishita H, et al.** Treatment of pancreatic fibrosis with siRNA against a collagen-specific chaperone in vitamin A-coupled liposomes. *Gut* 62(9): 1328-1339, 2013. PMID: 23172890.
38. **Jaster R, Lichte P, Fitzner B, Brock P, Glass A, Karopka T, et al.** Peroxisome proliferator-activated receptor gamma overexpression inhibits pro-fibrogenic activities of immortalised rat pancreatic stellate cells. *J Cell Mol Med* 9(3): 670-682, 2005. PMID: 16202214.
39. **Jones RJ, Gu D, Bjorklund CC, Kuiatse I, Remaley AT, Bashir T, et al.** The novel anticancer agent JNJ-26854165 induces cell death through inhibition of cholesterol transport and degradation of ABCA1. *J Pharmacol Exp Ther* 346(3): 381-392, 2013. PMID: 23820125.
40. **Kang HC, Nan JX, Park PH, Kim JY, Lee SH, Woo SW, et al.** Curcumin inhibits collagen synthesis and hepatic stellate cell activation in-vivo and in-vitro. *J Pharm Pharmacol* 54(1): 119-126, 2002. PMID: 11829122.
41. **Kaufmann KB, Buning H, Galy A, Schambach A and Grez M**. Gene therapy on the move. *EMBO Mol Med* 5(11): 1642-1661, 2013. PMID: 24106209.
42. **Khosla P, Patel V, Whinter JM, Khanna S, Rakhkovskaya M, Roy S, et al.** Postprandial levels of the natural vitamin E tocotrienol in human circulation. *Antioxid Redox Signal* 8(5-6): 1059-1068, 2006. PMID: 16771695.
43. **Lankisch PG, Breuer N, Bruns A, Weber-Dany B, Lowenfels AB and Maisonneuve P**. Natural history of acute pancreatitis: a long-term population-based study. *Am J Gastroenterol* 104(11): 2797-2805; quiz 2806, 2009. PMID: 19603011.
44. **Lee MS, Gu D, Feng L, Curriden S, Arnush M, Krahl T, et al.** Accumulation of extracellular matrix and developmental dysregulation in the pancreas by transgenic production of transforming growth factor-beta 1. *Am J Pathol* 147(1): 42-52, 1995. PMID: 7604884.
45. **Lerch MM and Gorelick FS**. Models of acute and chronic pancreatitis. *Gastroenterology* 144(6): 1180-1193, 2013. PMID: 23622127.
46. **Levy P, Dominguez-Munoz E, Imrie C, Lohr M and Maisonneuve P**. Epidemiology of chronic pancreatitis: burden of the disease and consequences. *United European Gastroenterol J* 2(5): 345-354, 2014. PMID: 25360312.
47. **Levy P, Milan C, Pignon JP, Baetz A and Bernades P**. Mortality factors associated with chronic pancreatitis. Unidimensional and multidimensional analysis of a medical-surgical series of 240 patients. *Gastroenterology* 96(4): 1165-1172, 1989. PMID: 2925060.
48. **Luttenberger T, Schmid-Kotsas A, Menke A, Siech M, Beger H, Adler G, et al.** Platelet-derived growth factors stimulate proliferation and extracellular matrix synthesis of pancreatic stellate cells: implications in pathogenesis of pancreas fibrosis. *Lab Invest* 80(1): 47-55, 2000. PMID: 10653002.
49. **Maleth J, Balazs A, Pallagi P, Balla Z, Kui B, Katona M, et al.** Alcohol disrupts levels and function of the cystic fibrosis transmembrane conductance regulator to promote development of pancreatitis. *Gastroenterology* 148(2): 427-439 e416, 2015. PMID: 25447846.
50. **Masamune A, Suzuki N, Kikuta K, Satoh M, Satoh K and Shimosegawa T**. Curcumin blocks activation of pancreatic stellate cells. *J Cell Biochem* 97(5): 1080-1093, 2006. PMID: 16294327.
51. **Massague J**. The transforming growth factor-beta family. *Annu Rev Cell Biol* 6: 597-641, 1990. PMID: 2177343.
52. **Matheson AJ and Figgitt DP**. Rofecoxib: a review of its use in the management of osteoarthritis, acute pain and rheumatoid arthritis. *Drugs* 61(6): 833-865, 2001. PMID: 11398914.
53. **Mayer JM, Kolodziej S, Jukka Laine V and Kahl S**. Immunomodulation in a novel model of experimental chronic pancreatitis. *Minerva Gastroenterol Dietol* 58(4): 347-354, 2012. PMID: 23207611.
54. **McCarroll JA, Phillips PA, Park S, Doherty E, Pirola RC, Wilson JS, et al.** Pancreatic stellate cell activation by ethanol and acetaldehyde: is it mediated by the mitogen-activated protein kinase signaling pathway? *Pancreas* 27(2): 150-160, 2003. PMID: 12883264.
55. **McCarroll JA, Phillips PA, Santucci N, Pirola RC, Wilson JS and Apte MV**. Vitamin A inhibits pancreatic stellate cell activation: implications for treatment of pancreatic fibrosis. *Gut* 55(1): 79-89, 2006. PMID: 16043492.

56. **McKay CJ, Glen P and McMillan DC**. Chronic inflammation and pancreatic cancer. *Best Pract Res Clin Gastroenterol* 22(1): 65-73, 2008. PMID: 18206813.
57. **Mews P, Phillips P, Fahmy R, Korsten M, Pirola R, Wilson J, et al.** Pancreatic stellate cells respond to inflammatory cytokines: potential role in chronic pancreatitis. *Gut* 50(4): 535-541, 2002. PMID: 11889076.
58. **Mrazek AA, Porro LJ, Bhatia V, Falzon M, Spratt H, Zhou J, et al.** Apigenin inhibits pancreatic stellate cell activity in pancreatitis. *J Surg Res* 196(1): 8-16, 2015. PMID: 25799526.
59. **Mukherjee R, Mareninova OA, Odinokova IV, Huang W, Murphy J, Chvanov M, et al.** Mechanism of mitochondrial permeability transition pore induction and damage in the pancreas: inhibition prevents acute pancreatitis by protecting production of ATP. *Gut*, 2015. PMID: 26071131.
60. **Muniraj T, Aslanian HR, Farrell J and Jamidar PA**. Chronic pancreatitis, a comprehensive review and update. Part I: epidemiology, etiology, risk factors, genetics, pathophysiology, and clinical features. *Dis Mon* 60(12): 530-550, 2014. PMID: 25510320.
61. **Nagashio Y, Ueno H, Imamura M, Asaumi H, Watanabe S, Yamaguchi T, et al.** Inhibition of transforming growth factor beta decreases pancreatic fibrosis and protects the pancreas against chronic injury in mice. *Lab Invest* 84(12): 1610-1618, 2004. PMID: 15502860.
62. **Nakatani K, Okuyama H, Shimahara Y, Saeki S, Kim DH, Nakajima Y, et al.** Cytoglobin/STAP, its unique localization in splanchnic fibroblast-like cells and function in organ fibrogenesis. *Lab Invest* 84(1): 91-101, 2004. PMID: 14647402.
63. **Nguyen MH and Wright TL**. Therapeutic advances in the management of hepatitis B and hepatitis C. *Curr Opin Infect Dis* 14(5): 593-601, 2001. PMID: 11964881.
64. **Niina Y, Ito T, Oono T, Nakamura T, Fujimori N, Igarashi H, et al.** A sustained prostacyclin analog, ONO-1301, attenuates pancreatic fibrosis in experimental chronic pancreatitis induced by dibutyltin dichloride in rats. *Pancreatology* 14(3): 201-210, 2014. PMID: 24854616.
65. **Nojgaard C**. Prognosis of acute and chronic pancreatitis - a 30-year follow-up of a Danish cohort. *Dan Med Bull* 57(12): B4228, 2010. PMID: 21122467.
66. **Olesen AE, Brokjaer A, Fisher IW and Larsen IM**. Pharmacological challenges in chronic pancreatitis. *World J Gastroenterol* 19(42): 7302-7307, 2013. PMID: 24259961.
67. **Omary MB, Lugea A, Lowe AW and Pandol SJ**. The pancreatic stellate cell: a star on the rise in pancreatic diseases. *J Clin Invest* 117(1): 50-59, 2007. PMID: 17200706.
68. **Patel V, Khanna S, Roy S, Ezziddin O and Sen CK**. Natural vitamin E alpha-tocotrienol: retention in vital organs in response to long-term oral supplementation and withdrawal. *Free Radic Res* 40(7): 763-771, 2006. PMID: 16984003.
69. **Pavan Kumar P, Radhika G, Rao GV, Pradeep R, Subramanyam C, Talukdar R, et al.** Interferon gamma and glycemic status in diabetes associated with chronic pancreatitis. *Pancreatology* 12(1): 65-70, 2012. PMID: 22487478.
70. **Pedrazzoli S, Pasquali C, Guzzinati S, Berselli M and Sperti C**. Survival rates and cause of death in 174 patients with chronic pancreatitis. *J Gastrointest Surg* 12(11): 1930-1937, 2008. PMID: 18766421.
71. **Pelli H, Sand J, Laippala P and Nordback I**. Long-term follow-up after the first episode of acute alcoholic pancreatitis: time course and risk factors for recurrence. *Scand J Gastroenterol* 35(5): 552-555, 2000. PMID: 10868461.
72. **Perluigi M, Di Domenico F and Butterfield DA**. mTOR signaling in aging and neurodegeneration: At the crossroad between metabolism dysfunction and impairment of autophagy. *Neurobiol Dis* 84: 39-49, 2015. PMID: 25796566.
73. **Punithavathi D, Venkatesan N and Babu M**. Curcumin inhibition of bleomycin-induced pulmonary fibrosis in rats. *Br J Pharmacol* 131(2): 169-172, 2000. PMID: 10991907.
74. **Qiu P, Feng XH and Li L**. Interaction of Smad3 and SRF-associated complex mediates TGF-beta1 signals to regulate SM22 transcription during myofibroblast differentiation. *J Mol Cell Cardiol* 35(12): 1407-1420, 2003. PMID: 14654367.
75. **Rajakumar DV and Rao MN**. Antioxidant properties of phenyl styryl ketones. *Free Radic Res* 22(4): 309-317, 1995. PMID: 7633561.
76. **Reding T, Bimmler D, Perren A, Sun LK, Fortunato F, Storni F, et al.** A selective COX-2 inhibitor suppresses chronic pancreatitis in an animal model (WBN/Kob rats): significant reduction of macrophage infiltration and fibrosis. *Gut* 55(8): 1165-1173, 2006. PMID: 16322109.
77. **Rickmann M, Vaquero EC, Malagelada JR and Molero X**. Tocotrienols induce apoptosis and autophagy in rat pancreatic stellate cells through the mitochondrial death pathway. *Gastroenterology* 132(7): 2518-2532, 2007. PMID: 17570223.
78. **Rinderknecht H**. Activation of pancreatic zymogens. Normal activation, premature intrapancreatic activation, protective mechanisms against inappropriate activation. *Dig Dis Sci* 31(3): 314-321, 1986. PMID: 2936587.

79. **Roberts AB, Russo A, Felici A and Flanders KC**. Smad3: a key player in pathogenetic mechanisms dependent on TGF-beta. *Ann N Y Acad Sci* 995: 1-10, 2003. PMID: 12814934.
80. **Sanvito F, Nichols A, Herrera PL, Huarte J, Wohlwend A, Vassalli JD, et al.** TGF-beta 1 overexpression in murine pancreas induces chronic pancreatitis and, together with TNF-alpha, triggers insulin-dependent diabetes. *Biochem Biophys Res Commun* 217(3): 1279-1286, 1995. PMID: 8554587.
81. **Sato Y, Murase K, Kato J, Kobune M, Sato T, Kawano Y, et al.** Resolution of liver cirrhosis using vitamin A-coupled liposomes to deliver siRNA against a collagen-specific chaperone. *Nat Biotechnol* 26(4): 431-442, 2008. PMID: 18376398.
82. **Scheller J, Garbers C and Rose-John S**. Interleukin-6: from basic biology to selective blockade of pro-inflammatory activities. *Semin Immunol* 26(1): 2-12, 2014. PMID: 24325804.
83. **Schlosser W, Schlosser S, Ramadani M, Gansauge F, Gansauge S and Beger HG**. Cyclooxygenase-2 is overexpressed in chronic pancreatitis. *Pancreas* 25(1): 26-30, 2002. PMID: 12131767.
84. **Schmid-Kotsas A, Gross HJ, Menke A, Weidenbach H, Adler G, Siech M, et al.** Lipopolysaccharide-activated macrophages stimulate the synthesis of collagen type I and C-fibronectin in cultured pancreatic stellate cells. *Am J Pathol* 155(5): 1749-1758, 1999. PMID: 10550331.
85. **Schmitz-Winnenthal H, Pietsch DH, Schimmack S, Bonertz A, Udonta F, Ge Y, et al.** Chronic pancreatitis is associated with disease-specific regulatory T-cell responses. *Gastroenterology* 138(3): 1178-1188, 2010. PMID: 19931255.
86. **Schneider A and Whitcomb DC**. Hereditary pancreatitis: a model for inflammatory diseases of the pancreas. *Best Pract Res Clin Gastroenterol* 16(3): 347-363, 2002. PMID: 12079262.
87. **Schuppan D, Krebs A, Bauer M and Hahn EG**. Hepatitis C and liver fibrosis. *Cell Death Differ* 10 Suppl 1: S59-67, 2003. PMID: 12655347.
88. **Sen CK, Khanna S and Roy S**. Tocotrienols: Vitamin E beyond tocopherols. *Life Sci* 78(18): 2088-2098, 2006. PMID: 16458936.
89. **Shen H, Zhang M, Minuk GY and Gong Y**. Different effects of rat interferon alpha, beta and gamma on rat hepatic stellate cell proliferation and activation. *BMC Cell Biol* 3: 9, 2002. PMID: 11940252.
90. **Shukla S and Gupta S**. Apigenin: a promising molecule for cancer prevention. *Pharm Res* 27(6): 962-978, 2010. PMID: 20306120.
91. **Siriwardena AK, Mason JM, Sheen AJ, Makin AJ and Shah NS**. Antioxidant therapy does not reduce pain in patients with chronic pancreatitis: the ANTICIPATE study. *Gastroenterology* 143(3): 655-663 e651, 2012. PMID: 22683257.
92. **Stark GR, Kerr IM, Williams BR, Silverman RH and Schreiber RD**. How cells respond to interferons. *Annu Rev Biochem* 67: 227-264, 1998. PMID: 9759489.
93. **Sugimoto K, Hanai H, Tozawa K, Aoshi T, Uchijima M, Nagata T, et al.** Curcumin prevents and ameliorates trinitrobenzene sulfonic acid-induced colitis in mice. *Gastroenterology* 123(6): 1912-1922, 2002. PMID: 12454848.
94. **Treiber M, Neuhofer P, Anetsberger E, Einwachter H, Lesina M, Rickmann M, et al.** Myeloid, but not pancreatic, RelA/p65 is required for fibrosis in a mouse model of chronic pancreatitis. *Gastroenterology* 141(4): 1473-1485, 1485 e1471-1477, 2011. PMID: 21763242.
95. **Trikudanathan G, Navaneethan U and Vege SS**. Modern treatment of patients with chronic pancreatitis. *Gastroenterol Clin North Am* 41(1): 63-76, 2012. PMID: 22341250.
96. **Tsang SW, Zhang H, Lin C, Xiao H, Wong M, Shang H, et al.** Rhein, a natural anthraquinone derivative, attenuates the activation of pancreatic stellate cells and ameliorates pancreatic fibrosis in mice with experimental chronic pancreatitis. *PLoS One* 8(12): e82201, 2013. PMID: 24312641.
97. **Tsujimoto T, Tsuruzono T, Hoppo K, Matsumura Y, Yamao J and Fukui H**. Effect of bromhexine hydrochloride therapy for alcoholic chronic pancreatitis. *Alcohol Clin Exp Res* 29(12 Suppl): 272S-276S, 2005. PMID: 16385235.
98. **Uden S, Bilton D, Nathan L, Hunt LP, Main C and Braganza JM**. Antioxidant therapy for recurrent pancreatitis: placebo-controlled trial. *Aliment Pharmacol Ther* 4(4): 357-371, 1990. PMID: 2103755.
99. **Vitone LJ, Greenhalf W, Howes NR and Neoptolemos JP**. Hereditary pancreatitis and secondary screening for early pancreatic cancer. *Rocz Akad Med Bialymst* 50: 73-84, 2005. PMID: 16358943.
100. **Vonlaufen A, Phillips PA, Xu Z, Zhang X, Yang L, Pirola RC, et al.** Withdrawal of alcohol promotes regression while continued alcohol intake promotes persistence of LPS-induced pancreatic injury in alcohol-fed rats. *Gut* 60(2): 238-246, 2011. PMID: 20870739.
101. **Wang J, Zhao H, Kong W, Jin C, Zhao Y, Qu Y, et al.** Microcalorimetric assay on the antimicrobial property of five hydroxyanthraquinone derivatives in rhubarb (Rheum palmatum L.) to Bifidobacterium adolescentis. *Phytomedicine* 17(8-9): 684-689, 2010. PMID: 19962872.

102. **Wen L, Voronina S, Javed MA, Awais M, Szatmary P, Latawiec D, et al.** Inhibitors of ORAI1 Prevent Cytosolic Calcium-Associated Injury of Human Pancreatic Acinar Cells and Acute Pancreatitis in 3 Mouse Models. *Gastroenterology* 149(2): 481-492 e487, 2015. PMID: 25917787.
103. **Wenzel S, Castro M, Corren J, Maspero J, Wang L, Zhang B, et al.** Dupilumab efficacy and safety in adults with uncontrolled persistent asthma despite use of medium-to-high-dose inhaled corticosteroids plus a long-acting beta2 agonist: a randomised double-blind placebo-controlled pivotal phase 2b dose-ranging trial. *Lancet*, 2016. PMID: 27130691.
104. **Westlund KN**. Gene therapy for pancreatitis pain. *Gene Ther* 16(4): 483-492, 2009. PMID: 19262610.
105. **Whitcomb DC**. Hereditary pancreatitis: new insights into acute and chronic pancreatitis. *Gut* 45(3): 317-322, 1999. PMID: 10446089.
106. **Whitcomb DC**. Mechanisms of disease: Advances in understanding the mechanisms leading to chronic pancreatitis. *Nat Clin Pract Gastroenterol Hepatol* 1(1): 46-52, 2004. PMID: 16265044.
107. **Whitcomb DC, Gorry MC, Preston RA, Furey W, Sossenheimer MJ, Ulrich CD, et al.** Hereditary pancreatitis is caused by a mutation in the cationic trypsinogen gene. *Nat Genet* 14(2): 141-145, 1996. PMID: 8841182.
108. **Whitcomb DC, LaRusch J, Krasinskas AM, Klei L, Smith JP, Brand RE, et al.** Common genetic variants in the CLDN2 and PRSS1-PRSS2 loci alter risk for alcohol-related and sporadic pancreatitis. *Nat Genet* 44(12): 1349-1354, 2012. PMID: 23143602.
109. **Wilcox CM, Yadav D, Ye T, Gardner TB, Gelrud A, Sandhu BS, et al.** Chronic pancreatitis pain pattern and severity are independent of abdominal imaging findings. *Clin Gastroenterol Hepatol* 13(3): 552-560; quiz e528-559, 2015. PMID: 25424572.
110. **Witt H, Apte MV, Keim V and Wilson JS**. Chronic pancreatitis: challenges and advances in pathogenesis, genetics, diagnosis, and therapy. *Gastroenterology* 132(4): 1557-1573, 2007. PMID: 17466744.
111. **Xue J, Sharma V and Habtezion A**. Immune cells and immune-based therapy in pancreatitis. *Immunol Res* 58(2-3): 378-386, 2014. PMID: 24710635.
112. **Xue J, Sharma V, Hsieh MH, Chawla A, Murali R, Pandol SJ, et al.** Alternatively activated macrophages promote pancreatic fibrosis in chronic pancreatitis. *Nat Commun* 6: 7158, 2015. PMID: 25981357.
113. **Yang DY, Fushimi H, Cai SQ and Komatsu K**. Molecular analysis of Rheum species used as Rhei Rhizoma based on the chloroplast matK gene sequence and its application for identification. *Biol Pharm Bull* 27(3): 375-383, 2004. PMID: 14993806.
114. **Yang X, Sun G, Yang C and Wang B**. Novel rhein analogues as potential anticancer agents. *ChemMedChem* 6(12): 2294-2301, 2011. PMID: 21954017.
115. **Yin H, Cabrera-Perez J, Lai Z, Michael D, Weller M, Swaim WD, et al.** Association of bone morphogenetic protein 6 with exocrine gland dysfunction in patients with Sjogren's syndrome and in mice. *Arthritis Rheum* 65(12): 3228-3238, 2013. PMID: 23982860.
116. **Zechner D, Knapp N, Bobrowski A, Radecke T, Genz B and Vollmar B**. Diabetes increases pancreatic fibrosis during chronic inflammation. *Exp Biol Med (Maywood)* 239(6): 670-676, 2014. PMID: 24719378.
117. **Zha M, Xu W, Zhai Q, Li F, Chen B and Sun Z**. High glucose aggravates the detrimental effects of pancreatic stellate cells on Beta-cell function. *Int J Endocrinol* 2014: 165612, 2014. PMID: 25097548.
118. **Zheng L, Xue J, Jaffee EM and Habtezion A**. Role of immune cells and immune-based therapies in pancreatitis and pancreatic ductal adenocarcinoma. *Gastroenterology* 144(6): 1230-1240, 2013. PMID: 23622132.
119. **Zhou CH, Lin L, Zhu XY, Wen T, Hu DM, Dong Y, et al.** Protective effects of edaravone on experimental chronic pancreatitis induced by dibutyltin dichloride in rats. *Pancreatology* 13(2): 125-132, 2013. PMID: 23561970.
120. **Zion O, Genin O, Kawada N, Yoshizato K, Roffe S, Nagler A, et al.** Inhibition of transforming growth factor beta signaling by halofuginone as a modality for pancreas fibrosis prevention. *Pancreas* 38(4): 427-435, 2009. PMID: 19188864.