## Title
KRAS, YAP, and obesity in pancreatic cancer: A signaling network with multiple loops



## Authors
Eibl, Guido
Rozengurt, Enrique

## Publication Date
2019-02-01

## DOI
10.1016/j.semcancer.2017.10.007

Peer reviewed

Published in final edited form as:

*Semin Cancer Biol*. 2019 February ; 54: 50–62. doi:10.1016/j.semcancer.2017.10.007.

# KRAS, YAP, and obesity in pancreatic cancer: a signaling network with multiple loops

**Guido Eibl**[1,3] and **Enrique Rozengurt**[2,3]

[1]Department of Surgery, David Geffen School of Medicine at UCLA, Los Angeles, California, United States

[2]Department of Medicine, David Geffen School of Medicine at UCLA, Los Angeles, California, United States

[3]CURE: Digestive Diseases Research Center, University of California at Los Angeles, Los Angeles, California, United States

## Abstract

Pancreatic ductal adenocarcinoma (PDAC) continues to be a lethal disease with no efficacious treatment modalities. The incidence of PDAC is expected to increase, at least partially because of the obesity epidemic. Increased efforts to prevent or intercept this disease are clearly needed. Mutations in *KRAS* are initiating events in pancreatic carcinogenesis supported by genetically engineered mouse models of the disease. However, oncogenic *KRAS* is not entirely sufficient for the development of fully invasive PDAC. Additional genetic mutations and/or environmental, nutritional, and metabolic stressors, e.g. inflammation and obesity, are required for efficient PDAC formation with activation of KRAS downstream effectors. Multiple factors “upstream” of KRAS associated with obesity, including insulin resistance, inflammation, changes in gut microbiota and GI peptides, can enhance/modulate downstream signals. Multiple signaling networks and feedback loops “downstream” of KRAS have been described that respond to obesogenic diets. We propose that *KRAS* mutations potentiate a signaling network that is promoted by environmental factors. Specifically, we envisage that *KRAS* mutations increase the intensity and duration of the growth-promoting signaling network. As the transcriptional activator YAP plays a critical role in the network, we conclude that the rationale for targeting the network (at different points), e.g. with FDA approved drugs such as statins and metformin, is therefore compelling.

### Keywords

Pancreatic cancer; oncogenic Kras; obesity; signaling network; YAP

Corresponding author: Guido Eibl, MD, Department of Surgery, David Geffen School of Medicine at UCLA, 72-236 CHS 10833 Le Conte Avenue, Los Angeles, CA 90095, Geibl@mednet.ucla.edu.

## 1. Importance of *KRAS* mutations in pancreatic cancer

Pancreatic cancer, of which pancreatic ductal adenocarcinoma (PDAC) represents the most common histological subtype, has been and continues to be an aggressive and lethal disease with an overall 5-year survival rate of only about 7% [1]. The worldwide estimates of its incidence and mortality in the general population are 8 cases per 100,000 person-years and 7 deaths per 100,000 person-years, and they are significantly higher in the United States than the rest of the world [2]. The incidence in the United States is estimated to increase to 53,670 new cases (27,970 males and 25,700 females) in 2017 and it is currently the fourth leading cause of cancer mortality in both men and women [1]. Despite advances in understanding the biology of PDAC, molecularly targeted therapy has not been translated into improved prognosis. In fact, total deaths due to PDAC are projected to rise to become the second leading cause of cancer-related deaths before 2030 [3]. Consequently, research efforts are increasingly focusing on prevention and interception, a novel concept, which encompasses halting transformed cells from becoming malignant [4–8]. The identification of modifiable risk factors and a better understanding of the molecular mechanisms of PDAC promotion will clearly guide the discovery of novel targets and agents for prevention.

Mutations in the *KRAS* oncogene were first associated with PDAC more than 30 years ago [9,10]. Since then numerous studies in humans and mice have confirmed the importance of *KRAS* mutations in the initiation of PDAC. Recent exome sequencing established *KRAS* to be the most frequently mutated gene in PDAC (~95%) [11,12]. Approximately 98% of all missense *KRAS* mutations in PDAC occur at position G12, with a G12D single amino acid substitution as the most prevalent [13]. Other missense mutations at position G13 (21%) or Q61 (28%) occur at lower frequency [13]. A recent integrated genomic characterization of PDAC using deep whole exome sequencing revealed multiple different *KRAS* mutations in a subset of tumors, with some PDACs showing evidence of biallelic mutations [14]. Furthermore, *KRAS* wild-type tumors were found to harbor mutations in other oncogenic drivers, including *GNAS* and *BRAF*, and additional RAS pathway genes, emphasizing the importance of *KRAS* and KRAS pathway genes in PDAC [14]. Missense mutations in G12 with single amino acid substitutions, which prevent interactions between KRAS and KRAS GTPase-activating proteins (GAPs), lead to constitutive activation of KRAS on a single molecule level. This has been thought to induce and sustain activation of a multitude of downstream signaling effectors, which ultimately result in many of the phenotypic hallmarks of cancer [13,15], including unhindered proliferation, suppression of apoptotic cell death, reprogramming of the cellular energy metabolism, evasion of immune system surveillance, and metastatic spread.

PDACs arise through a step-wise progression from precursor lesions, i.e. pancreatic intraepithelial neoplasias (PanINs) [16–19]. The discovery that over 90% of low-grade PanIN lesions harbor oncogenic *KRAS* mutations [20] led to the step-wise carcinogenesis paradigm, in which *KRAS* mutations are characterized as early, initiating events [13,21]. This notion is strongly supported by genetically engineered mouse models of PDAC [22–25]. The endogenous KRAS models, in which mutated *Kras* is expressed from its endogenous locus conditionally driven by PDX1 and PTF1-p48, transcription factors critically important for foregut (and pancreatic) differentiation, are among the most widely

used and considered state-of-the-art models [24,25]. This so-called KC mouse model closely recapitulates the human disease in terms of histopathological and genetic features, including the development and step-wise progression of PanINs [22]. Besides the importance of KRAS in PDAC initiation, more recently *Kras* mutations have been demonstrated to be also important for the maintenance of PDAC [26]. During early carcinogenesis inactivation of KRAS led to re-differentiation of PanINs to normal pancreatic lineages (acinar cells) or apoptosis, while inactivation of KRAS in advanced disease led to tumor regression [26,27]. However, in KC mouse models (without additional genetic alterations) the development of invasive PDAC occurs very late (usually after 9 months) and only in few animals (5–10%) [22]. Mutated *Kras* seems to be necessary but not sufficient for the development of invasive PDAC. The presence of another mutation, e.g. in the *Trp53* tumor suppressor gene, greatly accelerates PDAC development [23,28].

Importantly, environmental, nutritional, and metabolic factors, e.g. inflammation, obesity, type-2 diabetes mellitus (T2DM) also seem to be capable to promote PDAC development, potentially substituting additional genetic alterations as required factors for PDAC formation. This notion is supported by the use of genetically engineered mouse models. In the absence of additional promotional factors “upstream” of KRAS, expression of oncogenic *Kras* in mice at physiologic levels was capable to transforming only a small percentage of cells [29]. Signaling pathways downstream of KRAS, e.g. mitogen-activated protein kinase (MAPK), were not activated when oncogenic *Kras* was expressed from its endogenous locus in mice [30]. Similarly, incubation of human PDAC cells in a serum-free synthetic medium did no exhibit constitutive ERK pathway activation despite harboring *KRAS* activating mutations, though ERK could be stimulated by restoring growth factors [31–33]. Although expression of oncogenic *Kras* in adult mice failed to induce pancreatic neoplasia, a simultaneous pancreatic inflammation causes PanIN development and cancers [34]. Overall, the current available literature suggests that oncogenic *Kras* is necessary but not fully sufficient to transform cells and additional genetic or environmental factors might be required to raise the threshold of KRAS activity [27] to initiate and promote cancer development.

## 2. Obesity and type 2 diabetes mellitus as upstream factors of PDAC development

Among the known modifiable risk factors of PDAC are obesity and T2DM. There is strong epidemiologic evidence of an increased risk for PDAC in longstanding T2DM and new-onset diabetes mellitus [35–39]. About 85% of patients diagnosed with PDAC have impaired glucose tolerance or frank diabetes mellitus [37]. Meta-analyses have concluded that an about two-fold risk of PDAC exists in diabetic patients [40,41]. Possible mechanisms for increased PDAC risk in diabetic patients include cellular proliferative effects of hyperglycemia, hyperinsulinemia, and abnormalities in insulin/IGF receptor pathways. The importance of insulin in that context is underscored by reports showing an increased risk of PDAC in patients treated with insulin or insulin secretagogues [37] and by the link between hyperinsulinemia and cancer incidence [42]. Furthermore, diabetic patients treated with metformin have an improved insulin sensitivity and reduced risk of developing PDAC

further underscoring the importance of diabetes mellitus and hyperinsulinemia as risk factors for PDAC [37,43,44].

There is strong evidence that obesity is associated with an increased risk of cancer, including PDAC [45–52]. A recent umbrella review of systematic reviews and meta-analyses described that the association between obesity and PDAC is supported by strong evidence [53]. The International Agency for Research on Cancer reported a significant association between obesity and PDAC and described the strength of the currently available evidence as sufficient [54]. The projected increase in PDAC may thereby be related to the obesity endemic. In addition to an overall increased PDAC risk in obese people, our own data showed that obese people have an earlier onset of the disease. While the percentage of non-obese patients with onset of PDAC at the age of 50 years or younger (early-onset PDAC) was 6.1%, this percentage increased in obese and morbidly obese patients to 8.4% and 11.6%, respectively (unpublished).

Obesity and T2DM often coexist, but act independently to increase the risk for developing PDAC [55]. Obesity, as measured by an increased body mass index (BMI) was associated with an increased risk of death from several other cancers (such as esophagus, liver, and colon) [56–58] in which T2DM is less prevalent supporting an independent role of obesity in cancer development. It is important to note that the use of BMI to assess cancer risk might be oversimplified and often times inadequate because the distribution of fat appears to also influence cancer risk. Visceral obesity, as measured by an increased waist-to-hip ratio or waist circumference, has a stronger correlation to the metabolic syndrome and the development of certain cancers, including PDAC [48].

## 3. Animal models of obesity-associated PDAC

Genetically engineered animal models strongly confirmed the promotional effect of obesity on PDAC development. Using the KC mouse model we reported that a diet high in fats and calories (HFCD) led to substantial weight gain and accelerated early pancreatic neoplasia and invasive PDAC formation in obese mice [59,60]. No pancreatic neoplastic lesions were detected in lean or obese wildtype mice, underscoring the necessity of oncogenic *Kras* in PDAC development. Similar to humans, obese mice developed metabolic disturbances including hyperleptinemia, hyperinsulinemia, and elevated levels of insulin like growth factor-1 (IGF-1), indicating a level of insulin resistance [59]. Blood lipid levels, triglycerides and cholesterol, were not significantly different between obese and lean KC mice. Diet-induced obesity (DIO) in KC mice was associated with a robust inflammation of the visceral adipose tissue (VAT), as determined by an increase in crown-like structures (dying adipocytes surrounded by macrophages), by an elevated production of inflammatory cytokines, and by a greater number of inflammatory immune cells [61]. Similarly, DIO induced a strong inflammatory response in the pancreas, as indicated by an increase in the pancreatic inflammation index, a composite score of acinar cell loss, desmoplasia, and immune cell infiltration [59].

Molecularly, the pancreas of obese KC mice (compared to lean KC mice) showed increased activation of KRAS downstream pathways, including MAPK and PI3K/AKT/mTORC1. In

addition, the transcriptional activator YAP was more strongly expressed in the pancreas of obese KC mice (compared to lean KC mice), suggesting a responsiveness of the YAP pathway to environmental and metabolic stressors (unpublished data). The importance of YAP in PDAC will be discussed in detail in a later section below. Using KRAS G-LISA assays we did not detect significant differences of KRAS activity between pancreas samples from obese versus lean mice. Together these data suggest that DIO in the KC mouse model promotes PDAC development and progression by stimulating KRAS downstream signaling pathways rather than directly affecting KRAS activity.

In order to investigate whether DIO and the DIO-induced inflammatory microenvironment induce genetic alterations that could explain the acceleration of PDAC development in KC mice, we performed exome sequencing on laser-captured PanIN-2/3 lesions [60]. Exome sequencing of micro-dissected PanIN-2/3 lesions identified a total of more than 4,500 genetic variants specific to the obese, HFCD-fed group [60]. As expected, the *KrasG12D* mutation was detected in each of the PanIN lesions. There were no additional somatic mutations present within the *Kras* gene. Moreover, there were no somatic mutations detected in a panel of genes that are commonly mutated in human PDAC, including *Trp53*, *Smad4* and *Ink4a/Arf*. Using pathway analysis, we found several mutations in key molecules within the insulin signaling pathway, *e.g. mTor* and class II PI3K isoforms that may be of functional relevance [60]. Together, these sequencing results suggest that additional genetic mutations may explain at least some of the promotional effects of the HFCD and DIO on PDAC development in KC mice, although again no additional genetic variants in the most commonly mutated genes in PDAC were detected.

Others have also documented an increase in PDAC in high fat-fed and/or obese mice, thereby corroborating our data [62,63]. Mice that expressed oncogenic *Kras* in the adult pancreas under an acinar cell-specific promoter and were fed an isocaloric high fat diet gained significantly more body weight, developed enhanced pancreatic inflammation and showed accelerated PanIN progression [63]. This was associated with elevated activation of KRAS downstream signaling, e.g. ERK. The tumor promoting effect of the high fat diet was thereby reversed in a cyclooxygenase-2 (COX-2) deficient background, indicating the importance of inflammation in this process [63]. This is in agreement with our previous study, in which administration of a selective COX-2 inhibitor attenuated PDAC development in the KC mouse model [64]. In another study, mice with a pancreas-specific activation of oncogenic *Kras* fed a high fat diet showed accelerated PanIN progression [65]. In this case, the tumor promoting effects of the high fat diet were mediated by a low-grade systemic inflammation, as PanIN development was attenuated on a TNF-α receptor-deficient background. However, in this study the high fat diet led to pancreatic exocrine insufficiency and changes in energy metabolism, which contributed to an improved glucose tolerance. Mice on the high fat diet did not become obese and remained insulin sensitive. Differences in the genetic background of the animals might explain some of the discrepancies to our study. Overall, convincing evidence from preclinical mouse models exists demonstrating a promoting effect of high fat diet and DIO on PDAC development with enhanced activation of KRAS downstream effectors.

## 4. Extra-pancreatic mechanisms by which obesity and T2DM promote PDAC

The mechanisms, by which DIO promotes PDAC are multifaceted and highly interacting. In the subsequent sections, we divided these promotional processes in extra-pancreatic, i.e. processes that primarily occur outside the pancreas but impinge on the development of PDAC and pancreatic intracellular mechanisms (Figure 1). In what follows, we separated these processes to facilitate their discussion.

### Inflammation

A defining feature of obesity is the expansion of adipose tissue depots. Adipose tissue enlargement can occur by hyperplasia and/or hypertrophy of adipocytes. While hyperplasic adipose tissue expansion, which is seen primarily in subcutaneous adipose tissue, is considered “metabolically healthy”, adipocyte hypertrophy, commonly found in VAT, is associated with insulin resistance and inflammation [66,67]. Adipose tissue inflammation is characterized by profound changes in the number and function of resident and infiltrating immune cells, by the secretion of pro-inflammatory cytokines and adipokines, e.g. TNF-α, IL-1β, IL-6, MCP-1, leptin, and resistin, and by a decrease in anti-inflammatory molecules, e.g. IL-10, adiponectin [68]. Besides adipose tissue inflammation, obesity is also associated with a robust pancreatic inflammation [59,63]. Similar to adipose tissue inflammation, obesity-associated pancreatic inflammation occurs in the presence of profound changes in immune cell activity and cytokine production. It is unclear whether the pancreatic inflammation seen during obesity is simply a consequence of accompanying VAT inflammation or occurs independently from it. The observation that pancreatic inflammation (albeit to a lesser extent) is also seen in lean KC mice and is more robust in obese KC mice compared to obese wildtype mice indicates that the presence of oncogenic KRAS elicits some level of tissue inflammation independently of obesity-associated adipose tissue inflammation[59]. Overall, pro-inflammatory cytokines and adipokines may directly and indirectly affect PDAC development and growth, e.g. by modulating immune surveillance and cellular proliferation. Available data support the hypothesis that in the presence of oncogenic KRAS, inflammatory stimuli trigger a positive feedback mechanism, which involves NF-κB and COX-2 that amplifies KRAS activity to pathological levels leading to PDAC formation [30,63]. Mechanistically, we have previously reported that the COX-2 product prostaglandin E2 enhances mTORC-1 activity downstream of oncogenic KRAS in PDAC cells via a EP4/cAMP/PKA- and EP1/$Ca^{2+}$-mediated mechanism [69]. Recently, in interesting role of interleukin-17 (IL-17) has been described in PDAC [70,71]. IL-17, which contributes to obesity-associated inflammation in adipose tissue [72], promotes the transition from chronic pancreatitis to cancer in the context of oncogenic KRAS-driven PDAC[73]. In our own study, IL-17 was the most highly upregulated cytokine in the VAT of obese KC mice (unpublished data), suggesting an important IL-17-mediated positive reinforcement of oncogenic KRAS in obesity-associated PDAC. Taken together, the available data strongly suggest that tissue (adipose and pancreatic) inflammation, as seen during obesity, can enhance KRAS downstream signaling and promote/accelerate PDAC development.

## Gut microbiota

Obese animals and humans have altered gut microbiota when compared to their leaner counterparts. This alteration involves a greater representation of Firmicutes and fewer Bacteroides species in both mice and men. A reduced overall bacterial diversity as a whole and altered representation of bacterial genes are now considered a major cause for affecting metabolic pathways, which are likely involved in obesity [74]. Obesity-associated dysbiosis can result in several physiologic changes that may contribute to the relationship between obesity and cancer risk. These include i) altered microbial metabolism, which contributes to the generation of pro-carcinogenic metabolites, ii) increased extraction of energy and enhanced nutrient availability, and iii) induction of systemic low-grade inflammation initiating and promoting tumor development [75]. Gut microbiota, e.g. methanogens, ferment dietary polysaccharides resulting in the production of metabolites, namely monosaccharides and short-chain fatty acids (SCFAs), including butyrate, propionate, and acetate. These metabolites are absorbed and act as an energy source by the host. In addition, SCFAs can bind to intestinal free fatty acid receptors, including FFAR2 and FFAR3 that belong to the G protein-coupled receptor superfamily, and thereby regulate the secretion of gut hormones [76]. In obese individuals, compared to their lean counterparts, colonic fermentation patterns may be changed resulting in different fecal SCFA concentrations [76].

High-fat and high-energy diets have been shown to facilitate absorption of bacterial lipopolysaccharide (LPS), the most frequently studied pathogen-associated molecular pattern, from intestinal bacteria leading to a systemic inflammatory state through activation of toll-like receptor 4 [76,77]. A recent elegant study described that DIO altered gut microbiota with an increase in the production of deoxycholic acid (DCA), a gut bacterial metabolite known to cause DNA damage. The enterohepatic circulation of DCA induced a senescence-associated secretory phenotype (SASP) in the liver facilitating the development of hepatocellular carcinoma in mice [78]. Although studies on obesity, gut microbiome, and PDAC are rare, reports in other cancer models have shown that high fat diet-induced dysbiosis promoted oncogenic KRAS-driven intestinal tumorigenesis [79]. However, a recent meta-analysis questioned whether there are specific microbiome-based markers that can be associated with obesity [80]. The ability to reliably classify individuals as obese solely on the basis of their microbiome was limited due to a lack of power to detect modest effect sizes [80]. In conclusion, the possibility that metabolites produced by gut microbiota provide a mechanism linking dysbiosis to PDAC promotion via metabolite-sensing receptors acting either in pancreatic cells or in other cells, e.g. adipocytes, warrants further experimental work.

## Gastrointestinal (GI) peptides

Obesity modulates the production and release of GI hormones, including peptide YY (PYY), pancreatic polypeptide, ghrelin, glucagon peptide-1 (GL-1), cholecystokinin (CCK) and neurotensin (NT). These GI peptides are produced by specialized enteroendocrine cells located in the gut. These cells are thought to play a critical role in sensing the chemical composition of the luminal contents. An elegant study using NT-deficient mice implicated NT in the pathogenesis of obesity, hepatic steatosis, and insulin resistance associated with high fat consumption [81]. Interestingly, in humans both obese and insulin-resistant subjects

have elevated plasma concentrations of pro-NT, and in longitudinal studies among non-obese subjects, high levels of pro-NT denoted a doubling of the risk of developing obesity later in life [81]. These findings directly link the GI peptide NT with increased fat absorption and obesity and suggest that NT may provide a prognostic marker of future obesity and a potential target for prevention.

### Crosstalk between GPCRs and insulin/IGF-1 receptor signaling pathways

Most GI peptides initiate their characteristic cellular effects through heptahelical G protein-coupled receptors (GPCRs) in the surface of their target cells. Many studies support the notion that crosstalk between the insulin/IGF-1 receptor and GPCR signaling systems plays a key role in the regulation of normal and abnormal functions, including the pathogenesis of cardiovascular and renal pathologies in obesity and T2DM [82–90]. GPCRs and their cognate agonists also mediate autocrine/paracrine growth stimulation [91–98] and dramatically synergize with insulin/IGF-1 in inducing mitogenic signaling [99–105]. A recent characterization of cancer genomes demonstrated frequent mutations in GPCRs and G proteins [106]. Consequently, a crosstalk between insulin/IGF-1 receptor and GPCR signaling systems is a plausible mechanism for enhancing the development of PDAC in obesity and a potential target for chemoprevention [107–109]. In line with this proposition, PDAC cells express multiple growth-promoting GPCRs and their cognate agonists [32,33,110–120], including NT, angiotensin, and endothelin, and a broad-spectrum GPCR antagonist [121,122] inhibited the growth of PDAC cells [116]. Moreover, expression of multiple GPCRs, including the high-affinity NT receptor, is markedly increased in human PDAC tissues [98,123–126] and in PanINs [127]. Thus, GI peptides released from enteroendocrine cells in the gut can have important roles in mediating DIO as well as in promoting PDAC proliferation acting synergistically with insulin/IGF-1 in PDAC cells. We conclude that oncogenic KRAS in conjunction with multiple extra-pancreatic factors act additively and/or synergistically on the pancreas to elicit a complex signaling network that leads to PDAC development (Figure 1).

## 5. Pancreatic intracellular mechanisms by which KRAS, obesity, and T2DM promote PDAC

### PI3K/AKT/mTOR

A key pathway in KRAS and insulin/IGF1R signaling is PI3K/AKT leading to mTOR activation [128]. This signaling module plays a pivotal role in stimulating proliferation of PDAC cells [129], is activated in PDAC tissues [130,131], and limits catabolic processes, including autophagy [132]. Reciprocally, suppression of mTOR and IGF-1 has been associated with the antitumor actions of caloric restriction [133,134]. mTOR functions as a catalytic subunit in two distinct multi-protein complexes, mTORC1 and mTORC2 [135,136] both of which impinge on YAP regulation (Figure 2). mTORC1, characterized by Raptor phosphorylates and controls at least two regulators of protein synthesis, the 40S ribosomal protein subunit S6 kinase (S6K) and the inhibitor of protein synthesis 4E-binding protein 1, referred to as 4EBP1 [137–140]. mTORC1 is acutely inhibited by rapamycin whereas mTORC2, characterized by Rictor and Sin1, is not acutely inhibited by this agent [141,142].

The heterodimer of the tumor suppressor TSC2 (tuberin) and TSC1 (hamartin) represses mTORC1 signaling [143,144] by acting as the GTPase-activator protein for the small G protein Rheb, a potent activator of mTORC1 signaling in its GTP-bound state [145,146]. Phosphorylation of TSC2 by AKT and/or ERK/p90RSK uncouples TSC1/TSC2 from Rheb, leading to Rheb-GTP accumulation and mTORC1 activation. The importance of mTORC1 activation in human PDAC is highlighted by survival analysis showing that increased TSC2 expression is a favorable prognostic marker for patients with PDAC (our unpublished results).

The Rag GTPases (RAGA/B and RAGC/D), in conjunction with the adaptor Ragulator, activate mTORC1 in response to amino acids [147]. Phosphatase and tensin homologue (PTEN) opposes PI3K by degrading PIP3 to PIP2 thereby inactivating AKT and mTOR signaling [148]. Inactivation of p53, as seen during the progression of 50–70% of PDAC, potently up-regulates the insulin/IGF-1/mTORC1 pathway [149,150]. It is also relevant that YAP stimulates mTORC1 via downregulation of PTEN [151] and increased amino acid (leucine) transport [152,153]. In turn, mTORC1 activation leads to YAP accumulation through impaired autophagy [154] giving rise to an amplification loop. We will discuss YAP, a major downstream target of KRAS in PDAC, in a subsequent section.

## PKD/MEK/ERK

Many GPCRs activate G proteins of the Gq and G12 families, thereby stimulating isoforms of phospholipase C (PLC), identified as one of the “core” signaling pathways that undergo somatic alterations in nearly all PDAC [155]. PLCs produce second messengers that activate protein kinase C (PKC), which, in turn, phosphorylates and activates the protein kinase D (PKD) family [92,156–158]. The PKC/PKD axis induces MEK/ERK/p90RSK activation and thereby potentiates KRAS signaling in promoting cell proliferation [158–161]. PKD stimulates Rho activation as well as functions as a Rho effector [162,163], thereby establishing a positive feedback loop (Figure 2). PKDs are rapidly activated by GPCR agonists in PDAC cells [33,113,164,165], are over-expressed in PDAC tissues [166] and PKD over-expression promotes PDAC cell proliferation [167], invasion [168] and acinar to ductal trans-differentiation [169]. Furthermore, a PKD family inhibitor blocked PDAC cell growth *in vitro* and *in vivo* [166]. Interestingly, PKD1 provides a potential molecular link between obesity, insulin secretion and PDAC. Indeed, *PRKD1* (which encodes PKD1) has been identified as a risk gene for obesity [170] and PKD1 has been implicated in the regulation of insulin secretion from the β cells of the pancreas [171].

The activation of GPCRs by their cognate agonists also leads to mTORC1 stimulation through two converging mechanisms: EGFR (ErbB1,HER1) transactivation [172] and PKD/ERK pathway [109,135,172]. Consequently, mTORC1 and PKD/ERK are sites of convergence, crosstalk and amplification in the signaling network triggered by GPCRs, EGFR and insulin/IGF-1 receptor systems in PDAC cells. Importantly, these obesity-sensitive pathways reinforce KRAS signaling and in turn, *KRAS* mutations leading to KRAS activation potentiates the signal intensity emanating from the GPCRs, EGFR and insulin/IGF-1 receptor network in PDAC cells. It is plausible that this amplification loop can play a role in enhancing the strength of KRAS signaling, alluded to in previous sections.

Consequently, the identification of the downstream targets and gene regulatory programs of this signaling system is of fundamental significance and major translational interest in the mechanisms, by which obesity promotes KRAS-initiated PDAC development.

## 6. The Hippo/YAP pathway: a site of signal convergence and integration in PDAC

The highly conserved Hippo pathway, originally identified in *Drosophila*, is attracting intense attention as a key regulator of development, organ-size, tissue regeneration and tumorigenesis [173]. The YAP/TAZ pathway is as a central downstream pathway in KRAS, PI3K, mTORC1/2, EGFR and GPCR signaling [174–177], all of which play a critical role in PDAC and in its promotion by obesogenic diets.

### YAP/TAZ pathway

A serine/threonine kinase cascade wherein Mst1/2 kinases, in complex with Sav1, phosphorylate and activate Lats1/2, in complex with its regulatory protein MOB1/2 is a major pathway in the transduction of canonical Hippo signals [173]. As illustrated in the scheme shown in Figure 2, Lats1/2 phosphorylates the transcriptional co-activators Yes-Associated Protein (YAP) and WW-domain-containing Transcriptional co-Activator with PDZ-binding motif (TAZ), two major downstream effectors of the Hippo pathway and novel sensors of the mevalonate and glycolytic pathways [178–180]. Structurally, YAP and TAZ share nearly half of the overall amino acid sequence and have very similar topology. The phosphorylation of YAP at $Ser^{127}$ and $Ser^{397}$, highly conserved residues located within a consensus sequence phosphorylated by the Hippo kinases Lats1/2 (HXRXXS), restricts its cellular localization to the cytoplasm, inhibits its activity and reduces its stability.

In the absence of phosphorylation, YAP localizes to the nucleus where it binds and activates the TEA-domain DNA-binding transcription factors (TEAD 1–4) thereby stimulating the expression of a variety of genes (Figure 2), including Connective Tissue Growth Factor (*CTGF*), *BIRC5*, Cysteine-rich angiogenic inducer 61 (*CYR61*), Amphiregulin (*AREG*) and endothelin 2 (*EDN2*). *CTGF* is one of the best-characterized direct target gene of YAP that contains three putative YAP-TEAD binding sites (GGAATG) in its promoter region. The products of YAP/TEAD-regulated genes have a major impact on important cell processes, including shaping the micro-environment (CTGF), opposing apoptosis (survivin, the product of *BIRC 5*), mediating angiogenesis (CYR61), signaling communication between cancer cells and immune cells (CXCL5) and promoting autocrine/paracrine proliferation via EGFR (AREG) and GPCR (EDN2). CXCL5, a chemokine produced by PDAC cells [181], has been proposed to promote infiltration by polymorphonuclear myeloid-derived suppressor cells (MDSC) into cancer tissues. Furthermore, CYR61 regulates other developmental pathways activated in PDAC, including Sonic Hedgehog [182]. YAP/TAZ also orchestrates epithelial-to-mesenchymal transition (EMT) and reprogramming of malignant cells to a more undifferentiated state. Another study has linked chronic inflammation and mechanical cues from the microenvironment to YAP/TAZ for eliciting pathological responses in regenerating epithelia [183]. It is recognized that YAP/TAZ acts as a context-specific oncogene [175]. In addition to the Hippo pathway, YAP/TAZ localization and activity is controlled by a

multitude of signals that include those mediated by GPCRs, PKD, Rho and actin cytoskeleton, which rapidly stimulate its transcriptional co-activator activity [173,184].

## Regulation of the YAP/TAZ pathway in PDAC

The YAP/TAZ pathway assumes an added importance in PDAC given that YAP is a key downstream target of KRAS signaling required for acinar-to-ductal metaplasia (ADM) and PanIN progression into PDAC [185,186]. Interestingly, we found that obesity was associated with a marked decrease of intact acini in KC mice, suggesting that DIO also enhances ADM [60]. YAP induces ADM at least in part via JAK/signal transducer and activator of transcription-3 (STAT3) signaling [186]. Interestingly, DIO in KC mice induced a marked activation of STAT3, as indicated by increase phosphorylation of STAT3 at Tyr-705 [60]. YAP is also a major mediator of pro-oncogenic mutant p53 [187], resistance to RAF/MEK inhibitors [188] and chemotherapy in PDAC [189]. In the context of this article, it is relevant that Hippo signaling also functions in the regulation of adipocyte cell proliferation and differentiation [190,191] thus playing a role in obesity.

In view of the importance of the crosstalk between insulin/IGF-1 receptor and GPCR systems in the context of obesity and PDAC promotion (discussed above), we examined whether YAP acts as a downstream effector of crosstalk between insulin and NT in human PDAC cells [192]. This study focused on rapid regulation of YAP localization, phosphorylation and activity assessed by the expression of YAP/TEAD-regulated genes. Stimulation of PDAC cells with insulin and NT induced rapid nuclear import and dephosphorylation of YAP and markedly increased the expression of YAP/TEAD-regulated genes, including *CTGF*, *CYR61* and *CXCL5* through PI3K and PKD [192]. Accordingly, recent studies with other cell types demonstrated that PI3K inhibits the activity of the Hippo pathway [193,194] and PKD stimulates YAP activation [184]. Consequently, YAP emerges as a central node of transcriptional convergence in the crosstalk between insulin receptor and GPCR signaling systems and of critical importance in the proliferative response induced by growth-promoting agonists in PDAC cells.

In addition to the rapid regulation by multiple mechanisms and upstream pathways that feed into the YAP/TAZ pathway, including Rho, actin cytoskeletal organization, PI3K, PKD and GPCR signaling (Figure 2), it is important to emphasize that additional pathways and epigenetic events regulate the level of protein expression of YAP and TAZ. In this regard, the RAS pathway promotes YAP1 stability independently of the Hippo pathway through downregulation of the ubiquitin ligase complex substrate recognition factors SOCS5/6, thereby increasing YAP stability [195]. A recent study demonstrated that eIF5A (eukaryotic translation initiation factor 5A), which is up-regulated by KRAS in PDAC and in KC mice, increases the tyrosine kinase PEAK1. In turn, the eIF5A/PEAK1 axis enhances YAP expression [196]. As indicated in a previous section, we found that YAP was also more strongly expressed in the pancreas of obese KC mice (compared to lean KC mice), suggesting a responsiveness of the YAP pathway to DIO (our unpublished data). The pathways that link obesity to YAP/TAZ expression are of great interest and require further experimental work. It is also important that amplification and overexpression of YAP can substitute for oncogenic *Kras* in PDAC mouse models [197]. These studies raise the

important notion that YAP not only acts downstream of KRAS but also that its hyper-activation and expression can circumvent the need for oncogenic *Kras* in PDAC [198].

### The YAP/TAZ pathway as a prognostic marker in PDAC

We next turn our attention to the importance of YAP in human PDAC. A number of studies indicated that YAP and TAZ are overactive in PDAC patient tumor samples [197,199,200] and a new report identified YAP expression as an independent prognostic marker for overall survival of PDAC [201]. In order to extend this important conclusion, we used a recently published interactive open-access database (www.proteinatlas.org/pathology) [202] to perform correlation analyses based on mRNA expression levels of genes of the YAP pathway in PDAC tissue and the clinical outcome (survival) of the patients. We found that higher expression of either YAP or of the YAP/TEAD-regulated genes *AJUBA, ANLN, AREG*, *ARHGAP29, CCND1*, *CXCL5*, *EDN2, JAG1* and *NOTCH2* was significantly associated ($p<0.001$) with unfavorable prognosis in PDAC. A recent study showed that activator protein-1 (AP-1, dimer of JUN and FOS proteins) factors potentiate YAP/TAZ/TEAD-dependent gene expression [203]. Interestingly, expression of *FOSL1*, a component of AP-1, is also strongly associated ($p< 0.001$) with unfavorable prognosis in PDAC, as shown recently by others [204]. In addition to AREG, which functions in an autocrine manner, other EGFR ligands, including TGFα, epiregulin (EPEG), and heparin binding EGF (HBEGF) that stimulate YAP activity are also associated with shorter overall survival in PDAC. Conversely, high expression of PKA, which inhibits YAP activity via stimulation of LATS kinases [205], is associated with a favorable prognosis in PDAC, presumably via inhibition of YAP function. These new findings indicate that YAP, YAP/TEAD-regulated genes (*AJUBA, ANLN, AREG*, *ARHGAP29, CCND1*, *CXCL5*, *EDN2, JAG1* and *NOTCH2*), components of AP-1 that synergize with YAP (*FOSL1*), and growth factors that stimulate YAP activity are convincingly associated with unfavorable survival in PDAC whereas higher expression of a YAP inhibitory pathway (PKA) portends a favorable prognosis. All these associations, which were highly statistically significant ($p<0.001$), emphasize that hyper-activation of the YAP pathway correlates with poorer survival of PDAC patients.

## 7. Chemoprevention strategies targeting YAP in obesity-promoted PDAC

In the light of the new developments discussed so far, including preclinical studies, mechanistic connections and survival analysis, there is intense interest in targeting YAP/TAZ in PDAC chemoprevention. Although inhibition of the activity of transcription factors or their co-activators is a challenging strategy, recent preclinical and epidemiological evidence as well as our own results suggest new avenues to target YAP/TAZ activity via statins and metformin in PDAC and other malignancies (Figure 2).

### Statins and PDAC

Many studies showed that the mevalonate pathway is markedly up-regulated in several epithelial cancers via mutant p53 [206–208] and AKT/mTORC1 [208]. Statins are specific inhibitors of the 3-hydroxy-methylglutaryl (HMG) CoA reductase [209], the rate-limiting enzyme in the generation of mevalonate, the first step in the biosynthesis of isoprenoids,

leading to farnesyl pyrophosphate (FPP), geranylgeranyl pyrophosphate (GG-PP) and cholesterol. The transfer of the geranylgeranyl moiety to a COOH-terminal cysteine of Rho GTPases is critical for their function in signal transduction. In turn, active Rho (i.e. Rho-GTP) plays an essential role in YAP/TAZ activation in many cell types. Statins that are usually well tolerated and generally safe have been long used to treat hypercholesterolemia and prevent cardiovascular diseases. Although initially inconsistent, mounting epidemiological studies indicate that use of statins is associated with a reduced risk and beneficial effects in PDAC [210–215], especially in men [213,214]. For example, a large study showed that statin use was associated with a 34% reduced PDAC risk with a stronger association in male subjects [213]. In addition to their potential use in primary prevention, statins may improve the survival of patients after resection of primary PDAC [216–218], indicating a possible role of statins in secondary PDAC prevention. Epidemiological associations do not establish causation and should be interpreted with caution, but support the need of mechanistic studies and prospective clinical trials. A high-throughput screens of compounds capable of altering the subcellular localization of YAP led to the identification of the statins as potential YAP inhibitors [219]. Our studies with human and mouse PDAC cells also show that statins strikingly inhibited YAP nuclear localization, YAP/TEAD-regulated genes, proliferation, and colony formation by PDAC cells (unpublished data). Thus, converging epidemiological and preclinical studies indicate a protective effect of statins in PDAC, which is emerging as a novel area of translational research. Given the dismal prognosis of PDAC, a chemoprevention strategy for potentially vulnerable populations, including obese individuals with a family history of PDAC, should receive serious consideration.

## Metformin and PDAC

Metformin (1,1-dimethylbiguanide hydrochloride) is the most widely prescribed drug for treatment of T2DM worldwide [220,221]. The primary systemic effect of metformin is the lowering of blood glucose levels through reduced hepatic gluconeogenesis and improved insulin sensitivity by increasing glucose uptake in peripheral tissues, including skeletal muscles and adipose tissue [222]. Metformin also reduces the circulating levels of insulin and IGF-1 in both diabetic and non-diabetic patients [223,224]. Epidemiologically, metformin administration has been linked with reduced incidence, recurrence and mortality of cancer in diabetic patients [225–232], although a therapeutic efficacy of metformin is not universally seen in all studies [233], especially in advanced cases of cancer. Indeed, a recent major meta-analysis indicated that the effects of metformin depend on tumor stage, with marked improved survival in patients with locally advanced disease but not in patients with metastatic PDAC [230]. Accordingly, recent studies concluded that the survival of T2DM patients with resected PDAC was significantly higher in metformin users than in the diabetic patient that did not receive metformin, suggesting that metformin should be considered for secondary chemoprevention [234,235]. Although numerous preclinical and epidemiological studies support the notion that metformin is beneficial in early stage or resected PDAC, the mechanisms involved remain incompletely understood.

At the cellular level, metformin indirectly stimulates AMP–activated protein kinase (AMPK) activation [236], though other AMPK-independent mechanisms of action operate, especially

at high concentrations [237,238]. AMPK is a well-known sensor of cellular energy being activated when ATP concentrations decrease and 5′-AMP concentrations increase [221]. The tumor suppressor LKB-1/STK11 is the major kinase phosphorylating the AMPK activation loop [239]. STE20-related adaptor (STRAD), a co-factor that allosterically stimulates LKB-1 activity, is a favorable prognostic marker in pancreatic cancer. Metformin does not act directly on AMPK but inhibits complex I activity of the mitochondrial respiratory chain [240,241], resulting in reduced ATP synthesis and increase in cellular AMP and ADP thereby leading to AMPK activation. In mechanistic experiments with metformin or other inhibitors of mitochondrial respiration, including the natural alkaloid berberine, it is important to use physiological concentrations of glucose in the culture medium [242,243].

AMPK inhibits cell proliferation by suppressing mTORC1 function via at least three mechanisms. AMPK stimulates TSC2 function via phosphorylation on Ser-1345 [244–246], leading to accumulation of Rheb-GDP (the inactive form) and thereby to inhibition of mTORC1 activation. AMPK also inhibits mTORC1 by direct phosphorylation of Raptor (on Ser-722 and Ser-792), which disrupts its association with mTOR[247]. Insulin/IGF-1-induced mTORC1 activation is also attenuated by AMPK by direct phosphorylation of IRS-1 on Ser-794, a site that interferes with PI3K activation [248,249]. In PDAC cells, we demonstrated that metformin potently stimulates AMPK activation in cells cultured in physiological glucose [242,250] and inhibited mTORC1, ERK and mitogenic signaling via AMPK at low concentrations [242,250,251]. In line with these results, mitochondrial targeting of metformin greatly increases its growth-suppressive effects [252]. Metformin also inhibited the growth of PDAC xenografts [108,253] and recent results show that metformin is effective inhibitor of PDAC development in the KC model subjected to an obesogenic diet (our unpublished results). Berberine, a different AMPK activator that inhibits mitochondrial ATP synthesis, also inhibited mTORC1, ERK, DNA synthesis, proliferation and growth of PDAC xenografts [251].

Recently, it is becoming apparent that AMPK not only inhibits mTORC1 but also opposes the growth-promoting activity of YAP via different mechanisms, including direct phosphorylation of YAP at Ser-94 [254,255], a residue that plays a critical role in the coupling of YAP with TEAD. AMPK also phosphorylates HMG-CoA reductase at Ser-872 and inhibits its activity thereby interfering with mevalonic acid synthesis [256]. These studies imply a direct connection between cellular energy status, AMPK and YAP/TAZ function. In this context, we recently found that an obesogenic diet induced a marked increase in YAP expression in the pancreas of KC mice and that the administration of metformin prevented the increase in YAP (our unpublished results). Because statins and metformin interfere with YAP function through different mechanisms (Figure 2), it is conceivable that a combination of these agents synergistically suppresses YAP/TAZ activity and thereby exerts cancer-protective activity at low concentrations of each agent.

## Feedback loops and effect of pathway inhibitors

In recent years, it has become apparent that potent negative feedback loops regulate most signaling pathways and thereby fine-tune the output of the signaling network. For example,

mounting evidence indicates that the mTORC1/S6K and RAF/MEK/ERK pathways not only promote growth-promoting signaling but also mediate potent negative feedback loops that restrain upstream signaling through insulin/IGF-1 and EGFR and other tyrosine kinase receptors [257]. For example, the mTORC1/S6K axis inhibits IRS-1 function via phosphorylation of multiple residues, including Ser-636/639 by mTORC1 and Ser-307/636/1001 by S6K [258]. Suppression of these feedback loops by inhibitors of mTORC1/S6K or MEK/ERK causes compensatory over-activation of upstream signaling nodes, including PI3K, AKT, and ERK that potentially oppose the anti-proliferative effects of the inhibitors [250,257,259]. It is conceivable that the up-regulation of these pathways promotes YAP activation thereby leading to drug resistance. Similarly, statin resistance can develop during its administration because of an increase in the expression of *HMGCR*, which should be expected to be up-regulated by suppression of a negative feedback loop. A detailed understanding of these feedback mechanisms will allow the design of rational combinations of therapeutic agents to overcome drug resistance produced by compensatory activation of upstream pathways.

## 8. Conclusions and implications

PDAC is an aggressive and lethal disease with an overall 5-year survival rate of only about 7%. Although new therapies are clearly needed, considerable research efforts should be increasingly concentrated on PDAC prevention and interception. In this context, the identification of modifiable risk factors and a better understanding of the molecular mechanisms of PDAC promotion will clearly guide the discovery of novel targets and agents for prevention.

There is persuasive epidemiological evidence that obesity is a major risk factor for PDAC. It is also established that >90% of PDACs harbor a mutation of *KRAS* that increases the duration and intensity of its GTP bound state. However, it is also evident that *KRAS* mutation is necessary but not sufficient for PDAC development. While a number of additional mutations, typically in tumor suppressor genes (*Trp53*, *Smad4* and *Cdkn2a*), markedly accelerates PDAC development in mouse models, it is also evident that environmental conditions, including obesity and inflammation (Figure 1), also promote PDAC in KC mice. As discussed in a previous section, the development of obesity-associated genetically engineered mouse models to replicate the disease is a major advance to explore new strategies for chemoprevention.

Mechanistically, we propose that PI3K/mTORC1 and PKD/ERK are sites of convergence, crosstalk, and amplification in the signaling network triggered by GPCRs, EGFR and insulin/IGF-1 receptor systems in PDAC cells. Importantly, these obesity-sensitive pathways reinforce KRAS signaling and in turn, *KRAS* mutation leading to prolonged KRAS activation potentiates the signal intensity emanating from GPCRs, EGFR, and insulin/IGF-1 receptor network in PDAC cells by stimulating PI3K and ERK, which are well-established downstream targets of activated KRAS. We propose that a major new element in this amplification loop are the YAP/TAZ transcriptional co-activators, which further intensify positive feedback loops in the network (Figure 2). Importantly, GPCRs, EGFR, and insulin/IGF-1 receptor signaling rapidly stimulate YAP nuclear localization and activity whereas

KRAS enhances YAP protein expression. In turn, the output of YAP stimulates signaling via EGFR (*AREG*) and GPCR (*EDN2*), which further stimulate KRAS. YAP also leads to PDAC survival (*BIRC5*) and evasion of immune surveillance (*CXCL5*). Thus, rather than increasing the strength of KRAS signaling, as proposed by others [260], we envisage that *KRAS* mutations potentiate a signaling network that is promoted by environmental factors, including pro-inflammatory mediators, adipocyte-derived factors, and GI peptides. Specifically, we envisage that *KRAS* mutations increase the intensity and duration of the growth-promoting signaling network. The model can accommodate multiple experimental results, including the induction of PDAC in KC mice by mutations in other genes such as the recent findings showing that chronic inflammation in mice lacking p53 develop (inefficiently) PDAC with hyperactive YAP but without *KRAS* mutations [261]. As YAP plays a critical role in the network, we conclude that the rationale for targeting the network (at different points) is therefore compelling. As an initial approach to achieve this end, we propose FDA-approved drugs extensively used for preventing cardiovascular disease (statins) and treating T2DM (metformin) as plausible strategies for disrupting the network at multiple points, especially in obese patients predisposed to PDAC.

## Acknowledgments

NIH Grant P01CA163200 supported the work of the laboratory of G.E. The work in the laboratory of E.R. was supported by NIH Grants R01DK100405, P30DK41301 and P01CA163200 and by the Department of Veterans Affair Merit Award 1I01BX001473. Additional funding for G.E. and E.R. laboratories came from the Hirshberg Foundation for Pancreatic Cancer Research.

## References

1. Siegel RL, Miller KD, Jemal A. Cancer Statistics, 2017. CA Cancer J Clin. 2017; 67:7–30. [PubMed: 28055103]
2. Xiao AY, Tan MLY, Wu LM, Asrani VM, Windsor JA, Yadav D, et al. Global incidence and mortality of pancreatic diseases: a systematic review, meta-analysis, and meta-regression of population-based cohort studies. The Lancet Gastroenterology & Hepatology. 1:45–55.
3. Rahib L, Smith BD, Aizenberg R, Rosenzweig AB, Fleshman JM, Matrisian LM. Projecting Cancer Incidence and Deaths to 2030: The Unexpected Burden of Thyroid, Liver, and Pancreas Cancers in the United States. Cancer Res. 2014; 74:2913–21. [PubMed: 24840647]
4. Meyskens FL Jr, Mukhtar H, Rock CL, Cuzick J, Kensler TW, Yang CS, et al. Cancer Prevention: Obstacles, Challenges and the Road Ahead. J Natl Cancer Inst. 2016; 108
5. Kensler TW, Spira A, Garber JE, Szabo E, Lee JJ, Dong Z, et al. Transforming Cancer Prevention through Precision Medicine and Immune-oncology. Cancer Prev Res (Phila). 2016; 9:2–10. [PubMed: 26744449]
6. Albini A, DeCensi A, Cavalli F, Costa A. Cancer Prevention and Interception: A New Era for Chemopreventive Approaches. Clin Cancer Res. 2016
7. Umar A, Dunn BK, Greenwald P. Future directions in cancer prevention. Nat Rev Cancer. 2012; 12:835–48. [PubMed: 23151603]
8. Miller MS, Allen P, Brentnall TA, Goggins M, Hruban RH, Petersen GM, et al. Pancreatic Cancer Chemoprevention Translational Workshop: Meeting Report. Pancreas. 2016; 45:1080–91. [PubMed: 27518363]
9. Almoguera C, Shibata D, Forrester K, Martin J, Arnheim N, Perucho M. Most human carcinomas of the exocrine pancreas contain mutant c-K-ras genes. Cell. 1988; 53:549–54. [PubMed: 2453289]
10. Smit VT, Boot AJ, Smits AM, Fleuren GJ, Cornelisse CJ, Bos JL. KRAS codon 12 mutations occur very frequently in pancreatic adenocarcinomas. Nucleic Acids Res. 1988; 16:7773–82. [PubMed: 3047672]

11. Jones S, Zhang X, Parsons DW, Lin JC, Leary RJ, Angenendt P, et al. Core signaling pathways in human pancreatic cancers revealed by global genomic analyses. Science. 2008; 321:1801–6. [PubMed: 18772397]
12. Biankin AV, Waddell N, Kassahn KS, Gingras MC, Muthuswamy LB, Johns AL, et al. Pancreatic cancer genomes reveal aberrations in axon guidance pathway genes. Nature. 2012; 491:399–405. [PubMed: 23103869]
13. Bryant KL, Mancias JD, Kimmelman AC, Der CJ. KRAS: feeding pancreatic cancer proliferation. Trends Biochem Sci. 2014; 39:91–100. [PubMed: 24388967]
14. Cancer Genome Atlas Research Network. Electronic address aadhe, Cancer Genome Atlas Research N. Integrated Genomic Characterization of Pancreatic Ductal Adenocarcinoma. Cancer Cell. 2017; 32:185–203 e13. [PubMed: 28810144]
15. Pylayeva-Gupta Y, Grabocka E, Bar-Sagi D. RAS oncogenes: weaving a tumorigenic web. Nat Rev Cancer. 2011; 11:761–74. [PubMed: 21993244]
16. Hansel DE, Kern SE, Hruban RH. Molecular pathogenesis of pancreatic cancer. Annu Rev Genomics Hum Genet. 2003; 4:237–56. [PubMed: 14527303]
17. Hruban RH, Adsay NV, Albores-Saavedra J, Compton C, Garrett ES, Goodman SN, et al. Pancreatic intraepithelial neoplasia: a new nomenclature and classification system for pancreatic duct lesions. Am J Surg Pathol. 2001; 25:579–86. [PubMed: 11342768]
18. Maitra A, Fukushima N, Takaori K, Hruban RH. Precursors to invasive pancreatic cancer. Adv Anat Pathol. 2005; 12:81–91. [PubMed: 15731576]
19. Hruban RH, Goggins M, Parsons J, Kern SE. Progression model for pancreatic cancer. Clin Cancer Res. 2000; 6:2969–72. [PubMed: 10955772]
20. Kanda M, Matthaei H, Wu J, Hong SM, Yu J, Borges M, et al. Presence of somatic mutations in most early-stage pancreatic intraepithelial neoplasia. Gastroenterology. 2012; 142:730–3 e9. [PubMed: 22226782]
21. Deramaudt T, Rustgi AK. Mutant KRAS in the initiation of pancreatic cancer. Biochim Biophys Acta. 2005; 1756:97–101. [PubMed: 16169155]
22. Hingorani SR, Petricoin EF, Maitra A, Rajapakse V, King C, Jacobetz MA, et al. Preinvasive and invasive ductal pancreatic cancer and its early detection in the mouse. Cancer Cell. 2003; 4:437–50. [PubMed: 14706336]
23. Hingorani SR, Wang L, Multani AS, Combs C, Deramaudt TB, Hruban RH, et al. Trp53R172H and KrasG12D cooperate to promote chromosomal instability and widely metastatic pancreatic ductal adenocarcinoma in mice. Cancer Cell. 2005; 7:469–83. [PubMed: 15894267]
24. Hruban RH, Adsay NV, Albores-Saavedra J, Anver MR, Biankin AV, Boivin GP, et al. Pathology of genetically engineered mouse models of pancreatic exocrine cancer: consensus report and recommendations. Cancer Res. 2006; 66:95–106. [PubMed: 16397221]
25. Hruban RH, Rustgi AK, Brentnall TA, Tempero MA, Wright CV, Tuveson DA. Pancreatic cancer in mice and man: the Penn Workshop 2004. Cancer Res. 2006; 66:14–7. [PubMed: 16397208]
26. Collins MA, Bednar F, Zhang Y, Brisset JC, Galban S, Galban CJ, et al. Oncogenic Kras is required for both the initiation and maintenance of pancreatic cancer in mice. J Clin Invest. 2012; 122:639–53. [PubMed: 22232209]
27. di Magliano MP, Logsdon CD. Roles for KRAS in pancreatic tumor development and progression. Gastroenterology. 2013; 144:1220–9. [PubMed: 23622131]
28. Aguirre AJ, Bardeesy N, Sinha M, Lopez L, Tuveson DA, Horner J, et al. Activated Kras and Ink4a/Arf deficiency cooperate to produce metastatic pancreatic ductal adenocarcinoma. Genes Dev. 2003; 17:3112–26. [PubMed: 14681207]
29. Campbell PM, Singh A, Williams FJ, Frantz K, Ulku AS, Kelley GG, et al. Genetic and pharmacologic dissection of Ras effector utilization in oncogenesis. Methods Enzymol. 2006; 407:195–217. [PubMed: 16757325]
30. Daniluk J, Liu Y, Deng D, Chu J, Huang H, Gaiser S, et al. An NF-kappaB pathway-mediated positive feedback loop amplifies Ras activity to pathological levels in mice. J Clin Invest. 2012; 122:1519–28. [PubMed: 22406536]

31. Seufferlein T, Van Lint J, Liptay S, Adler G, Schmid RM. Transforming growth factor alpha activates Ha-Ras in human pancreatic cancer cells with Ki-ras mutations. Gastroenterology. 1999; 116:1441–52. [PubMed: 10348828]
32. Ryder NM, Guha S, Hines OJ, Reber HA, Rozengurt E. G protein-coupled receptor signaling in human ductal pancreatic cancer cells: Neurotensin responsiveness and mitogenic stimulation. J Cell Physiol. 2001; 186:53–64. [PubMed: 11147814]
33. Guha S, Rey O, Rozengurt E. Neurotensin Induces Protein Kinase C-dependent Protein Kinase D Activation and DNA Synthesis in Human Pancreatic Carcinoma Cell Line PANC-1. Cancer Res. 2002; 62:1632–40. [PubMed: 11912133]
34. Guerra C, Schuhmacher AJ, Canamero M, Grippo PJ, Verdaguer L, Perez-Gallego L, et al. Chronic pancreatitis is essential for induction of pancreatic ductal adenocarcinoma by K-Ras oncogenes in adult mice. Cancer Cell. 2007; 11:291–302. [PubMed: 17349585]
35. Sah RP, Nagpal SJ, Mukhopadhyay D, Chari ST. New insights into pancreatic cancer-induced paraneoplastic diabetes. Nat Rev Gastroenterol Hepatol. 2013; 10:423–33. [PubMed: 23528347]
36. Hart PA, Bellin MD, Andersen DK, Bradley D, Cruz-Monserrate Z, Forsmark CE, et al. Type 3c (pancreatogenic) diabetes mellitus secondary to chronic pancreatitis and pancreatic cancer. The Lancet Gastroenterology & Hepatology. 1:226–37.
37. Cui Y, Andersen DK. Diabetes and pancreatic cancer. Endocr Relat Cancer. 2012; 19:F9–F26. [PubMed: 22843556]
38. Fisher WE. Diabetes: risk factor for the development of pancreatic cancer or manifestation of the disease? World J Surg. 2001; 25:503–8. [PubMed: 11396427]
39. Tan J, You Y, Guo F, Xu J, Dai H, Bie P. Association of elevated risk of pancreatic cancer in diabetic patients: A systematic review and meta-analysis. Oncol Lett. 2017; 13:1247–55. [PubMed: 28454242]
40. Ben Q, Xu M, Ning X, Liu J, Hong S, Huang W, et al. Diabetes mellitus and risk of pancreatic cancer: A meta-analysis of cohort studies. Eur J Cancer. 2011; 47:1928–37. [PubMed: 21458985]
41. Li D, Tang H, Hassan MM, Holly EA, Bracci PM, Silverman DT. Diabetes and risk of pancreatic cancer: a pooled analysis of three large case-control studies. Cancer Causes Control. 2011; 22:189–97. [PubMed: 21104117]
42. Tsujimoto T, Kajio H, Sugiyama T. Association between hyperinsulinemia and increased risk of cancer death in nonobese and obese people: A population-based observational study. Int J Cancer. 2017; 141:102–11. [PubMed: 28390156]
43. Wang Z, Lai ST, Xie L, Zhao JD, Ma NY, Zhu J, et al. Metformin is associated with reduced risk of pancreatic cancer in patients with type 2 diabetes mellitus: a systematic review and meta-analysis. Diabetes Res Clin Pract. 2014; 106:19–26. [PubMed: 24837144]
44. Gong J, Robbins LA, Lugea A, Waldron RT, Jeon CY, Pandol SJ. Diabetes, pancreatic cancer, and metformin therapy. Front Physiol. 2014; 5:426. [PubMed: 25426078]
45. Renehan AG, Zwahlen M, Egger M. Adiposity and cancer risk: new mechanistic insights from epidemiology. Nat Rev Cancer. 2015; 15:484–98. [PubMed: 26205341]
46. Allott EH, Hursting SD. Obesity and cancer: mechanistic insights from transdisciplinary studies. Endocr Relat Cancer. 2015; 22:R365–86. [PubMed: 26373570]
47. Preziosi G, Oben JA, Fusai G. Obesity and pancreatic cancer. Surg Oncol. 2014; 23:61–71. [PubMed: 24746917]
48. Aune D, Greenwood DC, Chan DS, Vieira R, Vieira AR, Navarro Rosenblatt DA, et al. Body mass index, abdominal fatness and pancreatic cancer risk: a systematic review and non-linear dose-response meta-analysis of prospective studies. Ann Oncol. 2012; 23:843–52. [PubMed: 21890910]
49. Kaaks R, Kuhn T. Epidemiology: obesity and cancer–the evidence is fattening up. Nat Rev Endocrinol. 2014; 10:644–5. [PubMed: 25265978]
50. Park J, Morley TS, Kim M, Clegg DJ, Scherer PE. Obesity and cancer–mechanisms underlying tumour progression and recurrence. Nat Rev Endocrinol. 2014; 10:455–65. [PubMed: 24935119]
51. Kant P, Hull MA. Excess body weight and obesity–the link with gastrointestinal and hepatobiliary cancer. Nat Rev Gastroenterol Hepatol. 2011; 8:224–38. [PubMed: 21386810]
52. Goodwin PJ, Stambolic V. Impact of the obesity epidemic on cancer. Annu Rev Med. 2015; 66:281–96. [PubMed: 25423596]

53. Kyrgiou M, Kalliala I, Markozannes G, Gunter MJ, Paraskevaidis E, Gabra H, et al. Adiposity and cancer at major anatomical sites: umbrella review of the literature. BMJ. 2017; 356:j477. [PubMed: 28246088]
54. Lauby-Secretan B, Scoccianti C, Loomis D, Grosse Y, Bianchini F, Straif K, et al. Body Fatness and Cancer–Viewpoint of the IARC Working Group. N Engl J Med. 2016; 375:794–8. [PubMed: 27557308]
55. Eibl G, Cruz-Monserrate Z, Korc M, Petrov MS, Goodarzi MO, Fisher WE, et al. Diabetes Mellitus and Obesity as Risk Factors for Pancreatic Cancer. J Acad Nutr Diet. 2017
56. Dixon JL, Copeland LA, Zeber JE, MacCarthy AA, Reznik SI, Smythe WR, et al. Association between diabetes and esophageal cancer, independent of obesity, in the United States Veterans Affairs population. Dis Esophagus. 2016; 29:747–51. [PubMed: 26455587]
57. Aggarwal G, Kamada P, Chari ST. Prevalence of diabetes mellitus in pancreatic cancer compared to common cancers. Pancreas. 2013; 42:198–201. [PubMed: 23000893]
58. Yang WS, Va P, Bray F, Gao S, Gao J, Li HL, et al. The role of pre-existing diabetes mellitus on hepatocellular carcinoma occurrence and prognosis: a meta-analysis of prospective cohort studies. PLoS One. 2011; 6:e27326. [PubMed: 22205924]
59. Dawson DW, Hertzer K, Moro A, Donald G, Chang HH, Go VL, et al. High-fat, high-calorie diet promotes early pancreatic neoplasia in the conditional KrasG12D mouse model. Cancer Prev Res (Phila). 2013; 6:1064–73. [PubMed: 23943783]
60. Chang H-H, Moro A, Takakura K, Su H-Y, Mo A, Nakanishi M, et al. Incidence of pancreatic cancer is dramatically increased by a high fat, high calorie diet in KrasG12D mice. PLOS ONE. 2017; 12:e0184455. [PubMed: 28886117]
61. Hertzer KM, Xu M, Moro A, Dawson DW, Du L, Li G, et al. Robust Early Inflammation of the Peripancreatic Visceral Adipose Tissue During Diet-Induced Obesity in the KrasG12D Model of Pancreatic Cancer. Pancreas. 2016; 45:458–65. [PubMed: 26495779]
62. Lashinger LM, Harrison LM, Rasmussen AJ, Logsdon CD, Fischer SM, McArthur MJ, et al. Dietary energy balance modulation of Kras- and Ink4a/Arf+/−-driven pancreatic cancer: the role of insulin-like growth factor-I. Cancer Prev Res (Phila). 2013; 6:1046–55. [PubMed: 23980075]
63. Philip B, Roland CL, Daniluk J, Liu Y, Chatterjee D, Gomez SB, et al. A high-fat diet activates oncogenic Kras and COX2 to induce development of pancreatic ductal adenocarcinoma in mice. Gastroenterology. 2013; 145:1449–58. [PubMed: 23958541]
64. Funahashi H, Satake M, Dawson D, Huynh NA, Reber HA, Hines OJ, et al. Delayed progression of pancreatic intraepithelial neoplasia in a conditional Kras(G12D) mouse model by a selective cyclooxygenase-2 inhibitor. Cancer Res. 2007; 67:7068–71. [PubMed: 17652141]
65. Khasawneh J, Schulz MD, Walch A, Rozman J, Hrabe de Angelis M, Klingenspor M, et al. Inflammation and mitochondrial fatty acid beta-oxidation link obesity to early tumor promotion. Proc Natl Acad Sci U S A. 2009; 106:3354–9. [PubMed: 19208810]
66. Kusminski CM, Bickel PE, Scherer PE. Targeting adipose tissue in the treatment of obesity-associated diabetes. Nat Rev Drug Discov. 2016; 15:639–60. [PubMed: 27256476]
67. Sun K, Kusminski CM, Scherer PE. Adipose tissue remodeling and obesity. J Clin Invest. 2011; 121:2094–101. [PubMed: 21633177]
68. Singh RG, Pendharkar SA, Gillies NA, Miranda-Soberanis V, Plank LD, Petrov MS. Associations between circulating levels of adipocytokines and abdominal adiposity in patients after acute pancreatitis. Clin Exp Med. 2017
69. Chang HH, Young SH, Sinnett-Smith J, Chou CE, Moro A, Hertzer KM, et al. Prostaglandin E2 activates the mTORC1 pathway through an EP4/cAMP/PKA- and EP1/Ca2+-mediated mechanism in the human pancreatic carcinoma cell line PANC-1. Am J Physiol Cell Physiol. 2015; 309:C639–49. [PubMed: 26310818]
70. Cully M. Cancer: Targeting IL-17 in pancreatic cancer. Nat Rev Drug Discov. 2014; 13:493. [PubMed: 24981357]
71. McAllister F, Leach SD. Targeting IL-17 for pancreatic cancer prevention. Oncotarget. 2014; 5:9530–1. [PubMed: 25393980]

72. Caer C, Rouault C, Le Roy T, Poitou C, Aron-Wisnewsky J, Torcivia A, et al. Immune cell-derived cytokines contribute to obesity-related inflammation, fibrogenesis and metabolic deregulation in human adipose tissue. Sci Rep. 2017; 7:3000. [PubMed: 28592801]
73. Loncle C, Bonjoch L, Folch-Puy E, Lopez-Millan MB, Lac S, Molejon MI, et al. IL17 Functions through the Novel REG3beta-JAK2-STAT3 Inflammatory Pathway to Promote the Transition from Chronic Pancreatitis to Pancreatic Cancer. Cancer Res. 2015; 75:4852–62. [PubMed: 26404002]
74. Mishra AK, Dubey V, Ghosh AR. Obesity: An overview of possible role(s) of gut hormones, lipid sensing and gut microbiota. Metabolism. 2016; 65:48–65.
75. Rogers CJ, Prabhu KS, Vijay-Kumar M. The microbiome and obesity-an established risk for certain types of cancer. Cancer J. 2014; 20:176–80. [PubMed: 24855004]
76. Parekh PJ, Balart LA, Johnson DA. The Influence of the Gut Microbiome on Obesity, Metabolic Syndrome and Gastrointestinal Disease. Clin Transl Gastroenterol. 2015; 6:e91. [PubMed: 26087059]
77. Djuric Z. Obesity-associated cancer risk: the role of intestinal microbiota in the etiology of the host proinflammatory state. Transl Res. 2017; 179:155–67. [PubMed: 27522986]
78. Yoshimoto S, Loo TM, Atarashi K, Kanda H, Sato S, Oyadomari S, et al. Obesity-induced gut microbial metabolite promotes liver cancer through senescence secretome. Nature. 2013; 499:97–101. [PubMed: 23803760]
79. Schulz MD, Atay C, Heringer J, Romrig FK, Schwitalla S, Aydin B, et al. High-fat-diet-mediated dysbiosis promotes intestinal carcinogenesis independently of obesity. Nature. 2014; 514:508–12. [PubMed: 25174708]
80. Sze MA, Schloss PD. Looking for a Signal in the Noise: Revisiting Obesity and the Microbiome. MBio. 2016; 7
81. Li J, Song J, Zaytseva YY, Liu Y, Rychahou P, Jiang K, et al. An obligatory role for neurotensin in high-fat-diet-induced obesity. Nature. 2016; 533:411–5. [PubMed: 27193687]
82. Gavi S, Shumay E, Wang HY, Malbon CC. G-protein-coupled receptors and tyrosine kinases: crossroads in cell signaling and regulation. Trends Endocrinol Metab. 2006; 17:48–54. [PubMed: 16460957]
83. Velloso LA, Folli F, Perego L, Saad MJ. The multi-faceted cross-talk between the insulin and angiotensin II signaling systems. Diabetes Metab Res Rev. 2006; 22:98–107. [PubMed: 16389635]
84. Horiuchi M, Mogi M, Iwai M. Signaling crosstalk angiotensin II receptor subtypes and insulin. Endocr J. 2006; 53:1–5. [PubMed: 16543666]
85. Sowers JR. Insulin resistance and hypertension. Am J Physiol Heart Circ Physiol. 2004; 286:H1597–602. [PubMed: 15072967]
86. Kakoki M, Kizer CM, Yi X, Takahashi N, Kim HS, Bagnell CR, et al. Senescence-associated phenotypes in Akita diabetic mice are enhanced by absence of bradykinin B2 receptors. J Clin Invest. 2006; 116:1302–9. [PubMed: 16604193]
87. Kakoki M, Takahashi N, Jennette JC, Smithies O. Diabetic nephropathy is markedly enhanced in mice lacking the bradykinin B2 receptor. Proc Natl Acad Sci U S A. 2004; 101:13302–5. [PubMed: 15326315]
88. Aguilar D, Solomon SD. ACE inhibitors and angiotensin receptor antagonists and the incidence of new-onset diabetes mellitus : an emerging theme. Drugs. 2006; 66:1169–77. [PubMed: 16827595]
89. Haffner SM. Risk constellations in patients with the metabolic syndrome: epidemiology, diagnosis, and treatment patterns. Am J Med. 2006; 119:S3–9.
90. Li J, Song J, Zaytseva YY, Liu Y, Rychahou P, Jiang K, et al. An obligatory role for neurotensin in high-fat-diet-induced obesity. Nature. 2016
91. Rozengurt E. Growth factors and cell proliferation. Curr Opin Cell Biol. 1992; 4:161–5. [PubMed: 1318056]
92. Rozengurt E. Signal transduction pathways in the mitogenic response to G protein-coupled neuropeptide receptor agonists. J Cell Physiol. 1998; 177:507–17. [PubMed: 10092204]
93. Gutkind JS. Cell growth control by G protein-coupled receptors: from signal transduction to signal integration. Oncogene. 1998; 17:1331–42. [PubMed: 9779981]

94. Rozengurt E. Autocrine loops, signal transduction, and cell cycle abnormalities in the molecular biology of lung cancer. Curr Opin Oncol. 1999; 11:116–22. [PubMed: 10188076]
95. Rozengurt E, Walsh JH. Gastrin, CCK, signaling, and cancer. Annu Rev Physiol. 2001; 63:49–76. [PubMed: 11181948]
96. Heasley LE. Autocrine and paracrine signaling through neuropeptide receptors in human cancer. Oncogene. 2001; 20:1563–9. [PubMed: 11313903]
97. Rozengurt E. Neuropeptides as growth factors for normal and cancer cells. Trends Endocrinol Metabol. 2002; 13:128–34.
98. Arafat HA, Gong Q, Chipitsyna G, Rizvi A, Saa CT, Yeo CJ. Antihypertensives as novel antineoplastics: angiotensin-I-converting enzyme inhibitors and angiotensin II type 1 receptor blockers in pancreatic ductal adenocarcinoma. J Am Coll Surg. 2007; 204:996–1005. [PubMed: 17481528]
99. Rozengurt E, Legg A, Pettican P. Vasopressin stimulation of mouse 3T3 cell growth. Proc Natl Acad Sci US A. 1979; 76:1284–7.
100. Dicker P, Rozengurt E. Phorbol esters and vasopressin stimulate DNA synthesis by a common mechanism. Nature. 1980; 287:607–12. [PubMed: 7432481]
101. Rozengurt E, Sinnett-Smith J. Bombesin stimulation of DNA synthesis and cell division in cultures of Swiss 3T3 cells. Proc Natl Acad Sci USA. 1983; 80:2936–40. [PubMed: 6344074]
102. Rozengurt E. Early signals in the mitogenic response. Science. 1986; 234:161–6. [PubMed: 3018928]
103. Seufferlein T, Withers DJ, Rozengurt E. Reduced requirement of mitogen-activated protein kinase (MAPK) activity for entry into the S phase of the cell cycle in Swiss 3T3 fibroblasts stimulated by bombesin and insulin. J Biol Chem. 1996; 271:21471–7. [PubMed: 8702930]
104. Withers DJ, Seufferlein T, Mann D, Garcia B, Jones N, Rozengurt E. Rapamycin dissociates p70(S6K) activation from DNA synthesis stimulated by bombesin and insulin in Swiss 3T3 cells. J Biol Chem. 1997; 272:2509–14. [PubMed: 8999966]
105. Mann DJ, Higgins T, Jones NC, Rozengurt E. Differential control of cyclins D1 and D3 and the cdk inhibitor p27Kip1 by diverse signalling pathways in Swiss 3T3 cells. Oncogene. 1997; 14:1759–66. [PubMed: 9150381]
106. Kan Z, Jaiswal BS, Stinson J, Janakiraman V, Bhatt D, Stern HM, et al. Diverse somatic mutation patterns and pathway alterations in human cancers. Nature. 2010; 466:869–73. [PubMed: 20668451]
107. Kisfalvi K, Rey O, Young SH, Sinnett-Smith J, Rozengurt E. Insulin Potentiates Ca2+ Signaling and Phosphatidylinositol 4,5-Bisphosphate Hydrolysis Induced by Gq Protein-Coupled Receptor Agonists through an mTOR-Dependent Pathway. Endocrinology. 2007; 148:3246–57. [PubMed: 17379645]
108. Kisfalvi K, Eibl G, Sinnett-Smith J, Rozengurt E. Metformin disrupts crosstalk between G protein-coupled receptor and insulin receptor signaling systems and inhibits pancreatic cancer growth. Cancer Res. 2009; 69:6539–45. [PubMed: 19679549]
109. Rozengurt E, Sinnett-Smith J, Kisfalvi K. Crosstalk between Insulin/Insulin-like Growth Factor-1 Receptors and G Protein-Coupled Receptor Signaling Systems: A Novel Target for the Antidiabetic Drug Metformin in Pancreatic Cancer. Clin Cancer Res. 2010; 16:2505–11. [PubMed: 20388847]
110. Qin Y, Ertl T, Cai RZ, Halmos G, Schally AV. Inhibitory effect of bombesin receptor antagonist RC-3095 on the growth of human pancreatic cancer cells in vivo and in vitro. Cancer Res. 1994; 54:1035–41. [PubMed: 8313359]
111. Hirata M, Tsuchida A, Iwao T, Sasaki T, Matsubara K, Yamamoto S, et al. Cholecystokinin regulates the invasiveness of human pancreatic cancer cell lines via protein kinase C pathway. International Journal of Oncology. 1999; 14:1129–35. [PubMed: 10339669]
112. Ehlers RA, Zhang Y, Hellmich MR, Evers BM. Neurotensin-mediated activation of MAPK pathways and AP-1 binding in the human pancreatic cancer cell line, MIA PaCa-2. Biochem Biophys Res Commun. 2000; 269:704–8. [PubMed: 10720480]

113. Rey O, Yuan J, Rozengurt E. Intracellular redistribution of protein kinase D2 in response to G-protein-coupled receptor agonists. Biochem Biophys Res Commun. 2003; 302:817–24. [PubMed: 12646243]
114. Guha S, Lunn JA, Santiskulvong C, Rozengurt E. Neurotensin Stimulates Protein Kinase C-dependent Mitogenic Signaling in Human Pancreatic Carcinoma Cell Line PANC-1. Cancer Res. 2003; 63:2379–87. [PubMed: 12750255]
115. Kisfalvi K, Guha S, Rozengurt E. Neurotensin and EGF induce synergistic stimulation of DNA synthesis by increasing the duration of ERK signaling in ductal pancreatic cancer cells. J Cell Physiol. 2005; 202:880–90. [PubMed: 15389644]
116. Guha S, Eibl G, Kisfalvi K, Fan RS, Burdick M, Reber H, et al. Broad-spectrum G protein-coupled receptor antagonist, [D-Arg1,D-Trp5,7,9,Leu11]SP: a dual inhibitor of growth and angiogenesis in pancreatic cancer. Cancer Res. 2005; 65:2738–45. [PubMed: 15805273]
117. Young SH, Rozengurt E. Qdot nanocrystal conjugates conjugated to bombesin or ANG II label the cognate G protein-coupled receptor in living cells. Am J Physiol Cell Physiol. 2006; 290:C728–32. [PubMed: 16236822]
118. Cayrol C, Clerc P, Bertrand C, Gigoux V, Portolan G, Fourmy D, et al. Cholecystokinin-2 receptor modulates cell adhesion through beta 1-integrin in human pancreatic cancer cells. Oncogene. 2006; 25:4421–8. [PubMed: 16547500]
119. Kawasaki D, Emori Y, Eta R, Iino Y, Hamano H, Yoshinaga K, et al. Effect of Z-360, a novel orally active CCK-2/gastrin receptor antagonist on tumor growth in human pancreatic adenocarcinoma cell lines in vivo and mode of action determinations in vitro. Cancer Chemother Pharmacol. 2007
120. Smith JP, Verderame MF, Ballard EN, Zagon IS. Functional significance of gastrin gene expression in human cancer cells. Regul Pept. 2004; 117:167. [PubMed: 14749036]
121. Seckl MJ, Higgins T, Widmer F, Rozengurt E. [D-Arg1,D-Trp5,7,9,Leu11]substance P: a novel potent inhibitor of signal transduction and growth in vitro and in vivo in small cell lung cancer cells. Cancer Res. 1997; 57:51–4. [PubMed: 8988040]
122. Sinnett-Smith J, Santiskulvong C, Duque J, Rozengurt E. [D-Arg(1),D-Trp(5,7,9),Leu(11)]substance P inhibits bombesin-induced mitogenic signal transduction mediated by both G(q) and G(12) in Swiss 3T3 cells. J Biol Chem. 2000; 275:30644–52. [PubMed: 10880515]
123. Elek J, Pinzon W, Park KH, Narayanan R. Relevant genomics of neurotensin receptor in cancer. Anticancer Research. 2000; 20:53–8. [PubMed: 10769634]
124. Wang L, Friess H, Zhu Z, Graber H, Zimmermann A, Korc M, et al. Neurotensin receptor-1 mRNA analysis in normal pancreas and pancreatic disease. Clin Cancer Res. 2000; 6:566–71. [PubMed: 10690540]
125. Reubi JC, Waser B, Friess H, Bèuchler M, Laissue J. Neurotensin receptors: a new marker for human ductal pancreatic adenocarcinoma. Gut. 1998; 42:546–50. [PubMed: 9616318]
126. Alshoukr F, Rosant C, Maes V, Abdelhak J, Raguin O, Burg S, et al. Novel Neurotensin Analogues for Radioisotope Targeting to Neurotensin Receptor-Positive Tumors. Bioconjug Chem. 2009; 20:1602–10. [PubMed: 19610615]
127. Körner M, Waser B, Strobel O, Büchler M, Reubi JC. Neurotensin receptors in pancreatic ductal carcinomas. EJNMMI Research. 2015; 5:17. [PubMed: 25859423]
128. Taniguchi CM, Emanuelli B, Kahn CR. Critical nodes in signalling pathways: insights into insulin action. Nat Rev Mol Cell Biol. 2006; 7:85–96. [PubMed: 16493415]
129. Asano T, Yao Y, Shin S, McCubrey J, Abbruzzese JL, Reddy SA. Insulin receptor substrate is a mediator of phosphoinositide 3-kinase activation in quiescent pancreatic cancer cells. Cancer Res. 2005; 65:9164–8. [PubMed: 16230374]
130. Asano T, Yao Y, Zhu J, Li D, Abbruzzese JL, Reddy SA. The rapamycin analog CCI-779 is a potent inhibitor of pancreatic cancer cell proliferation. Biochem Biophys Res Commun. 2005; 331:295–302. [PubMed: 15845392]
131. Pham NA, Schwock J, Iakovlev V, Pond G, Hedley DW, Tsao MS. Immunohistochemical analysis of changes in signaling pathway activation downstream of growth factor receptors in pancreatic duct cell carcinogenesis. BMC Cancer. 2008; 8:43. [PubMed: 18254976]

132. Lee JW, Park S, Takahashi Y, Wang H-G. The Association of AMPK with ULK1 Regulates Autophagy. PLoS ONE. 2010; 5:e15394. [PubMed: 21072212]
133. Kopelovich L, Fay JR, Sigman CC, Crowell JA. The Mammalian Target of Rapamycin Pathway as a Potential Target for Cancer Chemoprevention. Cancer Epidemiology Biomarkers & Prevention. 2007; 16:1330.
134. Harvey AE, Lashinger LM, Hays D, Harrison LM, Lewis K, Fischer SM, et al. Calorie Restriction Decreases Murine and Human Pancreatic Tumor Cell Growth, Nuclear Factor-κB Activation, and Inflammation-Related Gene Expression in an Insulin-like Growth Factor-1–Dependent Manner. PLoS ONE. 2014; 9:e94151. [PubMed: 24804677]
135. Foster KG, Fingar DC. Mammalian Target of Rapamycin (mTOR): Conducting the Cellular Signaling Symphony. J Biol Chem. 2010; 285:14071–7. [PubMed: 20231296]
136. Laplante M, Sabatini DM. mTOR signaling at a glance. J Cell Sci. 2009; 122:3589–94. [PubMed: 19812304]
137. Um SH, D'Alessio D, Thomas G. Nutrient overload, insulin resistance, and ribosomal protein S6 kinase 1, S6K1. Cell Metab. 2006; 3:393–402. [PubMed: 16753575]
138. Ali SM, Sabatini DM. Structure of S6 Kinase 1 Determines whether Raptor-mTOR or Rictor-mTOR Phosphorylates Its Hydrophobic Motif Site. J Biol Chem. 2005; 280:19445–8. [PubMed: 15809305]
139. Long X, Muller F, Avruch J. TOR action in mammalian cells and in Caenorhabditis elegans. Curr Top Microbiol Immunol. 2004; 279:115–38. [PubMed: 14560955]
140. Armengol G, Rojo F, Castellvi J, Iglesias C, Cuatrecasas M, Pons B, et al. 4E-binding protein 1: a key molecular "funnel factor" in human cancer with clinical implications. Cancer Res. 2007; 67:7551–5. [PubMed: 17699757]
141. Wullschleger S, Loewith R, Hall MN. TOR signaling in growth and metabolism. Cell. 2006; 124:471–84. [PubMed: 16469695]
142. Inoki K, Guan KL. Complexity of the TOR signaling network. Trends Cell Biol. 2006; 16:206–12. [PubMed: 16516475]
143. Tee AR, Fingar DC, Manning BD, Kwiatkowski DJ, Cantley LC, Blenis J. Tuberous sclerosis complex-1 and -2 gene products function together to inhibit mammalian target of rapamycin (mTOR)-mediated downstream signaling. Proc Natl Acad Sci U S A. 2002; 99:13571–6. [PubMed: 12271141]
144. Manning BD, Tee AR, Logsdon MN, Blenis J, Cantley LC. Identification of the tuberous sclerosis complex-2 tumor suppressor gene product tuberin as a target of the phosphoinositide 3-kinase/akt pathway. Mol Cell. 2002; 10:151–62. [PubMed: 12150915]
145. Garami A, Zwartkruis FJ, Nobukuni T, Joaquin M, Roccio M, Stocker H, et al. Insulin activation of Rheb, a mediator of mTOR/S6K/4E-BP signaling, is inhibited by TSC1 and 2. Mol Cell. 2003; 11:1457–66. [PubMed: 12820960]
146. Zhang Y, Gao X, Saucedo LJ, Ru B, Edgar BA, Pan D. Rheb is a direct target of the tuberous sclerosis tumour suppressor proteins. Nat Cell Biol. 2003; 5:578–81. [PubMed: 12771962]
147. Bar-Peled L, Sabatini DM. Regulation of mTORC1 by amino acids. Trends Cell Biol. 2014; 24:400–6. [PubMed: 24698685]
148. Song MS, Salmena L, Pandolfi PP. The functions and regulation of the PTEN tumour suppressor. Nat Rev Mol Cell Biol. 2012; 13:283–96. [PubMed: 22473468]
149. Feng Z. p53 Regulation of the IGF-1/AKT/mTOR Pathways and the Endosomal Compartment. Cold Spring Harb Perspect Biol. 2010; 2:a001057. [PubMed: 20182617]
150. Feng Z, Levine AJ. The regulation of energy metabolism and the IGF-1/mTOR pathways by the p53 protein. Trends Cell Biol. 2010; 20:427–34. [PubMed: 20399660]
151. Tumaneng K, Schlegelmilch K, Russell RC, Yimlamai D, Basnet H, Mahadevan N, et al. YAP mediates crosstalk between the Hippo and PI (3)K–TOR pathways by suppressing PTEN via miR-29. Nat Cell Biol. 2012; 14:1322–9. [PubMed: 23143395]
152. Park Y-Y, Sohn BH, Johnson RL, Kang M-H, Kim SB, Shim J-J, et al. Yes-associated protein 1 and transcriptional coactivator with PDZ-binding motif activate the mammalian target of rapamycin complex 1 pathway by regulating amino acid transporters in hepatocellular carcinoma. Hepatology. 2016; 63:159–72. [PubMed: 26389641]

153. Hansen CG, Ng YLD, Lam W-LM, Plouffe SW, Guan K-L. The Hippo pathway effectors YAP and TAZ promote cell growth by modulating amino acid signaling to mTORC1. Cell Res. 2015; 25:1299–313. [PubMed: 26611634]

154. Liang N, Zhang C, Dill P, Panasyuk G, Pion D, Koka V, et al. Regulation of YAP by mTOR and autophagy reveals a therapeutic target of tuberous sclerosis complex. J Exp Med. 2014; 211:2249–63. [PubMed: 25288394]

155. Olive KP, Jacobetz MA, Davidson CJ, Gopinathan A, McIntyre D, Honess D, et al. Inhibition of Hedgehog Signaling Enhances Delivery of Chemotherapy in a Mouse Model of Pancreatic. Cancer Science. 2009; 324:1457–61.

156. Exton JH. Regulation of phosphoinositide phospholipases by hormones, neurotransmitters, and other agonists linked to G proteins. Annu Rev Pharmacol Toxicol. 1996; 36:481–509. [PubMed: 8725399]

157. Berridge MJ, Lipp P, Bootman MD. The versatility and universality of calcium signalling. Nat Rev Mol Cell Biol. 2000; 1:11–21. [PubMed: 11413485]

158. Rozengurt E, Rey O, Waldron RT. Protein Kinase D Signaling. J Biol Chem. 2005; 280:13205–8. [PubMed: 15701647]

159. Sinnett-Smith J, Zhukova E, Hsieh N, Jiang X, Rozengurt E. Protein kinase D potentiates DNA synthesis induced by Gq-coupled receptors by increasing the duration of ERK signaling in swiss 3T3 cells. J Biol Chem. 2004; 279:16883–93. [PubMed: 14963034]

160. Sinnett-Smith J, Jacamo R, Kui R, Wang YM, Young SH, Rey O, et al. Protein Kinase D Mediates Mitogenic Signaling by Gq-coupled Receptors through Protein Kinase C-independent Regulation of Activation Loop Ser744 and Ser748 Phosphorylation. J Biol Chem. 2009; 284:13434–45. [PubMed: 19289471]

161. Zhukova E, Sinnett-Smith J, Rozengurt E. Protein Kinase D Potentiates DNA Synthesis and Cell Proliferation Induced by Bombesin, Vasopressin, or Phorbol Esters in Swiss 3T3 Cells. J Biol Chem. 2001; 276:40298–305. [PubMed: 11514571]

162. Yuan J, Slice LW, Rozengurt E. Activation of Protein Kinase D by Signaling through Rho and the alpha Subunit of the Heterotrimeric G Protein G13. J Biol Chem. 2001; 276:38619–27. [PubMed: 11507098]

163. Rozengurt E. Protein Kinase D Signaling: Multiple Biological Functions in Health and Disease. Physiology. 2011; 26:23–33. [PubMed: 21357900]

164. Yuan J, Rozengurt E. PKD, PKD2, and p38 MAPK mediate Hsp27 serine-82 phosphorylation induced by neurotensin in pancreatic cancer PANC-1 cells. J Cell Biochem. 2008; 103:648–62. [PubMed: 17570131]

165. Rey O, Yuan J, Young SH, Rozengurt E. Protein kinase C nu/protein kinase D3 nuclear localization, catalytic activation, and intracellular redistribution in response to G protein-coupled receptor agonists. J Biol Chem. 2003; 278:23773–85. [PubMed: 12676944]

166. Harikumar KB, Kunnumakkara AB, Ochi N, Tong Z, Deorukhkar A, Sung B, et al. A Novel Small-Molecule Inhibitor of Protein Kinase D Blocks Pancreatic Cancer Growth In vitro and In vivo. Mol Cancer Ther. 2010; 9:1136–46. [PubMed: 20442301]

167. Kisfalvi K, Hurd C, Guha S, Rozengurt E. Induced overexpression of protein kinase D1 stimulates mitogenic signaling in human pancreatic carcinoma PANC-1 cells. J Cell Physiol. 2010; 223:309–16. [PubMed: 20082306]

168. Ochi N, Tanasanvimon S, Matsuo Y, Tong Z, Sung B, Aggarwal BB, et al. Protein kinase D1 promotes anchorage-independent growth, invasion, and angiogenesis by human pancreatic cancer cells. J Cell Physiol. 2011; 226:1074–81. [PubMed: 20857418]

169. Liou G-Y, Döppler H, Braun UB, Panayiotou R, Scotti Buzhardt M, Radisky DC, et al. Protein kinase D1 drives pancreatic acinar cell reprogramming and progression to intraepithelial neoplasia. Nat Commun. 2015; 6

170. Schwenk RW, Vogel H, Schürmann A. Genetic and epigenetic control of metabolic health(). Mol Metab. 2013; 2:337–47. [PubMed: 24327950]

171. Sumara G, Formentini I, Collins S, Sumara I, Windak R, Bodenmiller B, et al. Regulation of PKD by the MAPK p38delta in insulin secretion and glucose homeostasis. Cell. 2009; 136:235–48. [PubMed: 19135240]

172. Rozengurt E. Mitogenic signaling pathways induced by G protein-coupled receptors. J Cell Physiol. 2007; 213:589–602. [PubMed: 17786953]

173. Meng Z, Moroishi T, Guan K-L. Mechanisms of Hippo pathway regulation. Genes Dev. 2016; 30:1–17. [PubMed: 26728553]

174. Yu F-X, Guan K-L. The Hippo pathway: regulators and regulations. Genes Dev. 2013; 27:355–71. [PubMed: 23431053]

175. Moroishi T, Hansen CG, Guan K-L. The emerging roles of YAP and TAZ in cancer. Nat Rev Cancer. 2015; 15:73–9. [PubMed: 25592648]

176. Straßburger K, Tiebe M, Pinna F, Breuhahn K, Teleman AA. Insulin/IGF signaling drives cell proliferation in part via Yorkie/YAP. Dev Biol. 2012; 367:187–96. [PubMed: 22609549]

177. Wang J, Sinnett-Smith J, Stevens JV, Young SH, Rozengurt E. Biphasic regulation of Yes-associated Protein (YAP) cellular localization, phosphorylation and activity by G protein-coupled receptor agonists in intestinal epithelial cells: a novel role for protein kinase D (PKD). J Biol Chem. 2016

178. Enzo E, Santinon G, Pocaterra A, Aragona M, Bresolin S, Forcato M, et al. Aerobic glycolysis tunes YAP/TAZ transcriptional activity. EMBO J. 2015; 34:1349–70. [PubMed: 25796446]

179. Wang Z, Wu Y, Wang H, Zhang Y, Mei L, Fang X, et al. Interplay of mevalonate and Hippo pathways regulates RHAMM transcription via YAP to modulate breast cancer cell motility. Proc Natl Acad Sci U S A. 2014; 111:E89–E98. [PubMed: 24367099]

180. Santinon G, Pocaterra A, Dupont S. Control of YAP/TAZ Activity by Metabolic and Nutrient-Sensing Pathways. Trends Cell Biol. 2016; 26:289–99. [PubMed: 26750334]

181. Li A, King J, Moro A, Sugi MD, Dawson DW, Kaplan J, et al. Overexpression of CXCL5 Is Associated With Poor Survival in Patients With Pancreatic Cancer. Am J Pathol. 2011; 178:1340–9. [PubMed: 21356384]

182. Haque I, De A, Majumder M, Mehta S, McGregor D, Banerjee SK, et al. The Matricellular Protein CCN1/Cyr61 Is a Critical Regulator of Sonic Hedgehog in Pancreatic Carcinogenesis. J Biol Chem. 2012; 287:38569–79. [PubMed: 23027863]

183. Nowell CS, Odermatt PD, Azzolin L, Hohnel S, Wagner EF, Fantner GE, et al. Chronic inflammation imposes aberrant cell fate in regenerating epithelia through mechanotransduction. Nat Cell Biol. 2016; 18:168–80. [PubMed: 26689676]

184. Wang J, Sinnett-Smith J, Stevens JV, Young SH, Rozengurt E. Biphasic Regulation of Yes-associated Protein (YAP) Cellular Localization, Phosphorylation, and Activity by G Protein-coupled Receptor Agonists in Intestinal Epithelial Cells: A NOVEL ROLE FOR PROTEIN KINASE D (PKD). J Biol Chem. 2016; 291:17988–8005. [PubMed: 27369082]

185. Zhang W, Nandakumar N, Shi Y, Manzano M, Smith A, Graham G, et al. Downstream of Mutant KRAS, the Transcription Regulator YAP Is Essential for Neoplastic Progression to Pancreatic Ductal Adenocarcinoma. Sci Signal. 2014; 7

186. Gruber R, Panayiotou R, Nye E, Spencer-Dene B, Stamp G, Behrens A. YAP1 and TAZ Control Pancreatic Cancer Initiation in Mice by Direct Up-regulation of JAK–STAT3 Signaling. Gastroenterology. 2016; 151:526–39. [PubMed: 27215660]

187. Di Agostino S, Sorrentino G, Ingallina E, Valenti F, Ferraiuolo M, Bicciato S, et al. YAP enhances the pro-proliferative transcriptional activity of mutant p53 proteins. EMBO Rep. 2016; 17:188–201. [PubMed: 26691213]

188. Lin L, Sabnis AJ, Chan E, Olivas V, Cade L, Pazarentzos E, et al. The Hippo effector YAP promotes resistance to RAF- and MEK-targeted cancer therapies. Nat Genet. 2015; 47:250–6. [PubMed: 25665005]

189. Neesse A, Frese KK, Bapiro TE, Nakagawa T, Sternlicht MD, Seeley TW, et al. CTGF antagonism with mAb FG-3019 enhances chemotherapy response without increasing drug delivery in murine ductal pancreas cancer. Proc Natl Acad Sci U S A. 2013; 110:12325–30. [PubMed: 23836645]

190. An Y, Kang Q, Zhao Y, Hu X, Li N. Lats2 Modulates Adipocyte Proliferation and Differentiation via Hippo Signaling. PLOS ONE. 2013; 8:e72042. [PubMed: 23977200]

191. Huang H, Wu W, Zhang L, Liu X-Y. Drosophila Ste-20 Family Protein Kinase, Hippo, Modulates Fat Cell Proliferation. PLOS ONE. 2013; 8:e61740. [PubMed: 23637896]

192. Hao F, Xu Q, Zhao Y, Stevens JV, Young SH, Sinnett-Smith J, et al. Insulin Receptor and GPCR Crosstalk Stimulates YAP via PI3K and PKD in Pancreatic Cancer Cells. Mol Cancer Res. 2017; 15:929–41. [PubMed: 28360038]

193. Fan R, Kim N-G, Gumbiner BM. Regulation of Hippo pathway by mitogenic growth factors via phosphoinositide 3-kinase and phosphoinositide-dependent kinase-1. Proc Natl Acad Sci U S A. 2013; 110:2569–74. [PubMed: 23359693]

194. Kim N-G, Gumbiner BM. Adhesion to fibronectin regulates Hippo signaling via the FAK–Src–PI3K pathway. J Cell Biol. 2015; 210:503–15. [PubMed: 26216901]

195. Hong X, Nguyen HT, Chen Q, Zhang R, Hagman Z, Voorhoeve PM, et al. Opposing activities of the Ras and Hippo pathways converge on regulation of YAP protein turnover. EMBO J. 2014; 33:2447. [PubMed: 25180228]

196. Strnadel J, Choi S, Fujimura K, Wang H, Zhang W, Wyse M, et al. eIF5A-PEAK1 Signaling Regulates YAP1/TAZ Protein Expression and Pancreatic Cancer Cell Growth. Cancer Res. 2017; 77:1997. [PubMed: 28381547]

197. Kapoor A, Yao W, Ying H, Hua S, Liewen A, Wang Q, et al. Yap1 Activation Enables Bypass of Oncogenic Kras Addiction in Pancreatic Cancer. Cell. 2014; 158:185–97. [PubMed: 24954535]

198. Ying H, Dey P, Yao W, Kimmelman AC, Draetta GF, Maitra A, et al. Genetics and biology of pancreatic ductal adenocarcinoma. Genes Dev. 2016; 30:355–85. [PubMed: 26883357]

199. Yang S, Zhang L, Purohit V, Shukla SK, Chen X, Yu F, et al. Active YAP promotes pancreatic cancer cell motility, invasion and tumorigenesis in a mitotic phosphorylation-dependent manner through LPAR3. 2015

200. Xie D, Cui J, Xia T, Jia Z, Wang L, Wei W, et al. Hippo transducer TAZ promotes epithelial mesenchymal transition and supports pancreatic cancer progression. 2015

201. Salcedo Allende MT, Zeron-Medina J, Hernandez J, Macarulla T, Balsells J, Merino X, et al. Overexpression of Yes Associated Protein 1, an Independent Prognostic Marker in Patients With Pancreatic Ductal Adenocarcinoma, Correlated With Liver Metastasis and Poor Prognosis. Pancreas. 2017; 46:913–20. [PubMed: 28697132]

202. Uhlen M, Zhang C, Lee S, Sjöstedt E, Fagerberg L, Bidkhori G, et al. A pathology atlas of the human cancer transcriptome. Science. 2017; 357

203. Zanconato F, Forcato M, Battilana G, Azzolin L, Quaranta E, Bodega B, et al. Genome-wide association between YAP/TAZ/TEAD and AP-1 at enhancers drives oncogenic growth. Nat Cell Biol. 2015; 17:1218–27. [PubMed: 26258633]

204. Vallejo A, Perurena N, Guruceaga E, Mazur PK, Martinez-Canarias S, Zandueta C, et al. An integrative approach unveils FOSL1 as an oncogene vulnerability in KRAS-driven lung and pancreatic cancer. Nat Commun. 2017; 8:14294. [PubMed: 28220783]

205. Kim M, Kim M, Lee S, Kuninaka S, Saya H, Lee H, et al. cAMP/PKA signalling reinforces the LATS–YAP pathway to fully suppress YAP in response to actin cytoskeletal changes. EMBO J. 2013; 32:1543–55. [PubMed: 23644383]

206. Freed-Pastor William A, Mizuno H, Zhao X, Langerød A, Moon S-H, Rodriguez-Barrueco R, et al. Mutant p53 Disrupts Mammary Tissue Architecture via the Mevalonate Pathway. Cell. 2012; 148:244–58. [PubMed: 22265415]

207. Clendening JW, Pandyra A, Boutros PC, Ghamrasni SE, Khosravi F, Trentin GA, et al. Dysregulation of the mevalonate pathway promotes transformation. Proc Natl Acad Sci U S A. 2010; 107:15051–6. [PubMed: 20696928]

208. Kuzu OF, Noory MA, Robertson GP. The Role of Cholesterol in Cancer. Cancer Res. 2016; 76:2063–70. [PubMed: 27197250]

209. Istvan ES, Deisenhofer J. Structural Mechanism for Statin Inhibition of HMG-CoA Reductase. Science. 2001; 292:1160–4. [PubMed: 11349148]

210. Jeon CY, Pandol SJ, Wu B, Cook-Wiens G, Gottlieb RA, Merz NB, et al. The Association of Statin Use after Cancer Diagnosis with Survival in Pancreatic Cancer Patients: A SEER-Medicare Analysis. PLoS ONE. 2015; 10:e0121783. [PubMed: 25830309]

211. Wu BU, Chang J, Jeon CY, Pandol SJ, Huang B, Ngor EW, et al. Impact of Statin Use on Survival in Patients Undergoing Resection for Early-Stage Pancreatic Cancer. Am J Gastroenterol. 2015; 110:1233–9. [PubMed: 26195180]

212. Chen M-J, Tsan Y-T, Liou J-M, Lee Y-C, Wu M-S, Chiu H-M, et al. Statins and the risk of pancreatic cancer in Type 2 diabetic patients—A population-based cohort study. Int J Cancer. 2016; 138:594–603. [PubMed: 26296262]

213. Walker EJ, Ko AH, Holly EA, Bracci PM. Statin use and risk of pancreatic cancer: Results from a large, clinic-based case-control study. Cancer. 2015; 121:1287–94. [PubMed: 25649483]

214. Carey FJ, Little MW, Pugh TFG, Ndokera R, Ing H, Clark A, et al. The Differential Effects of Statins on the Risk of Developing Pancreatic Cancer: A Case–Control Study in Two Centres in the United Kingdom. Dig Dis Sci. 2013; 58:3308–12. [PubMed: 23864194]

215. E J-Y, Lu S-E, Lin Y, Graber JM, Rotter D, Zhang L, et al. Differential and Joint Effects of Metformin and Statins on Overall Survival of Elderly Patients with Pancreatic Adenocarcinoma: A Large Population-Based Study. Cancer Epidemiol Biomarkers Prev. 2017; 26:1225. [PubMed: 28619830]

216. Jeon CY, Pandol SJ, Wu B, Cook-Wiens G, Gottlieb RA, Merz CN, et al. The association of statin use after cancer diagnosis with survival in pancreatic cancer patients: a SEER-medicare analysis. PLoS One. 2015; 10:e0121783. [PubMed: 25830309]

217. Wu BU, Chang J, Jeon CY, Pandol SJ, Huang B, Ngor EW, et al. Impact of statin use on survival in patients undergoing resection for early-stage pancreatic cancer. Am J Gastroenterol. 2015; 110:1233–9. [PubMed: 26195180]

218. Lee HS, Lee SH, Lee HJ, Chung MJ, Park JY, Park SW, et al. Statin Use and Its Impact on Survival in Pancreatic Cancer Patients. Medicine (Baltimore). 2016; 95:e3607. [PubMed: 27175667]

219. Sorrentino G, Ruggeri N, Specchia V, Cordenonsi M, Mano M, Dupont S, et al. Metabolic control of YAP and TAZ by the mevalonate pathway. Nat Cell Biol. 2014; 16:357–66. [PubMed: 24658687]

220. Witters LA. The blooming of the French lilac. J Clin Invest. 2001; 108:1105–7. [PubMed: 11602616]

221. Kahn BB, Alquier T, Carling D, Hardie DG. AMP-activated protein kinase: ancient energy gauge provides clues to modern understanding of metabolism. Cell Metab. 2005; 1:15–25. [PubMed: 16054041]

222. Shaw RJ, Lamia KA, Vasquez D, Koo SH, Bardeesy N, Depinho RA, et al. The kinase LKB1 mediates glucose homeostasis in liver and therapeutic effects of metformin. Science. 2005; 310:1642–6. [PubMed: 16308421]

223. Berker B, Emral R, Demirel C, Corapcioglu D, Unlu C, Kose K. Increased insulin-like growth factor-I levels in women with polycystic ovary syndrome, and beneficial effects of metformin therapy. Gynecol Endocrinol. 2004; 19:125–33. [PubMed: 15697073]

224. Goodwin P, Pritchard K, Ennis M, Clemons M, Graham M, Fantus IG. Insulin-Lowering Effects of Metformin in Women with Early Breast Cancer. Clinical Breast Cancer. 2008; 8:501. [PubMed: 19073504]

225. Evans JM, Donnelly LA, Emslie-Smith AM, Alessi DR, Morris AD. Metformin and reduced risk of cancer in diabetic patients. Bmj. 2005; 330:1304–5. [PubMed: 15849206]

226. Li D, Yeung SC, Hassan MM, Konopleva M, Abbruzzese JL. Antidiabetic therapies affect risk of pancreatic cancer. Gastroenterology. 2009; 137:482–8. [PubMed: 19375425]

227. Libby G, Donnelly LA, Donnan PT, Alessi DR, Morris AD, Evans JM. New users of metformin are at low risk of incident cancer: a cohort study among people with type 2 diabetes. Diabetes Care. 2009; 32:1620–5. [PubMed: 19564453]

228. Ben Sahra I, Le Marchand-Brustel Y, Tanti JF, Bost F. Metformin in cancer therapy: a new perspective for an old antidiabetic drug? Mol Cancer Ther. 2010; 9:1092–9. [PubMed: 20442309]

229. Sadeghi N, Abbruzzese JL, Yeung SC, Hassan M, Li D. Metformin use is associated with better survival of diabetic patients with pancreatic cancer. Clin Cancer Res. 2012; 18:2905–12. [PubMed: 22465831]

230. Li X, Li T, Liu Z, Gou S, Wang C. The effect of metformin on survival of patients with pancreatic cancer: a meta-analysis. Sci Rep. 2017; 7:5825. [PubMed: 28724893]

231. Lega IC, Shah PS, Margel D, Beyene J, Rochon PA, Lipscombe LL. The effect of metformin on mortality following cancer among patients with diabetes. Cancer Epidemiol Biomarkers Prev. 2014; 23:1974–84. [PubMed: 25030683]

232. Amin S, Mhango G, Lin J, Aronson A, Wisnivesky J, Boffetta P, et al. Metformin Improves Survival in Patients with Pancreatic Ductal Adenocarcinoma and Pre-Existing Diabetes: A Propensity Score Analysis. Am J Gastroenterol. 2016; 111:1350–7. [PubMed: 27430290]

233. Suissa S, Azoulay L. Metformin and cancer: mounting evidence against an association. Diabetes Care. 2014; 37:1786–8. [PubMed: 24963109]

234. Il Jang W, Kim M-S, Kang SH, Jo AJ, Kim YJ, Tchoe HJ, et al. Association between metformin use and mortality in patients with type 2 diabetes mellitus and localized resectable pancreatic cancer: a nationwide population-based study in korea. Oncotarget. 2017; 8:9587–96. [PubMed: 28077783]

235. Cerullo M, Gani F, Chen SY, Canner J, Pawlik TM. Metformin Use Is Associated with Improved Survival in Patients Undergoing Resection for Pancreatic Cancer. J Gastrointest Surg. 2016; 20:1572–80. [PubMed: 27255657]

236. Hawley SA, Ross FA, Chevtzoff C, Green KA, Evans A, Fogarty S, et al. Use of cells expressing gamma subunit variants to identify diverse mechanisms of AMPK activation. Cell Metab. 2010; 11:554–65. [PubMed: 20519126]

237. Sahra IB, Laurent K, Loubat A, Giorgetti-Peraldi S, Colosetti P, Auberger P, et al. The antidiabetic drug metformin exerts an antitumoral effect in vitro and in vivo through a decrease of cyclin D1 level. Oncogene. 2008; 27:3576. [PubMed: 18212742]

238. Kalender A, Selvaraj A, Kim SY, Gulati P, Brulé S, Viollet B, et al. Metformin, Independent of AMPK, Inhibits mTORC1 in a Rag GTPase-Dependent Manner. Cell Metabol. 2010; 11:390–401.

239. Hezel AF, Bardeesy N. LKB1; linking cell structure and tumor suppression. Oncogene. 2008; 27:6908–19. [PubMed: 19029933]

240. Owen MR, Doran E, Halestrap AP. Evidence that metformin exerts its anti-diabetic effects through inhibition of complex 1 of the mitochondrial respiratory chain. Biochem J. 2000; 348:607–14. [PubMed: 10839993]

241. El-Mir MY, Nogueira V, Fontaine E, Averet N, Rigoulet M, Leverve X. Dimethylbiguanide inhibits cell respiration via an indirect effect targeted on the respiratory chain complex I. J Biol Chem. 2000; 275:223–8. [PubMed: 10617608]

242. Sinnett-Smith J, Kisfalvi K, Kui R, Rozengurt E. Metformin inhibition of mTORC1 activation, DNA synthesis and proliferation in pancreatic cancer cells: dependence on glucose concentration and role of AMPK. Biochem Biophys Res Commun. 2013; 430:352–3527. [PubMed: 23159620]

243. Rozengurt E. Mechanistic target of rapamycin (mTOR): a point of convergence in the action of insulin/IGF-1 and G protein-coupled receptor agonists in pancreatic cancer cells. Front Physiol. 2014; 5

244. Inoki K, Zhu T, Guan KL. TSC2 mediates cellular energy response to control cell growth and survival. Cell. 2003; 115:577–90. [PubMed: 14651849]

245. Shaw RJ, Bardeesy N, Manning BD, Lopez L, Kosmatka M, DePinho RA, et al. The LKB1 tumor suppressor negatively regulates mTOR signaling. Cancer Cell. 2004; 6:91–9. [PubMed: 15261145]

246. Inoki K, Ouyang H, Zhu T, Lindvall C, Wang Y, Zhang X, et al. TSC2 integrates Wnt and energy signals via a coordinated phosphorylation by AMPK and GSK3 to regulate cell growth. Cell. 2006; 126:955–68. [PubMed: 16959574]

247. Gwinn DM, Shackelford DB, Egan DF, Mihaylova MM, Mery A, Vasquez DS, et al. AMPK Phosphorylation of Raptor Mediates a Metabolic Checkpoint. Molecular Cell. 2008; 30:214–26. [PubMed: 18439900]

248. Tzatsos A, Tsichlis PN. Energy depletion inhibits phosphatidylinositol 3-kinase/Akt signaling and induces apoptosis via AMP-activated protein kinase-dependent phosphorylation of IRS-1 at Ser-794. J Biol Chem. 2007; 282:18069–82. [PubMed: 17459875]

249. Ning J, Clemmons DR. AMP-Activated Protein Kinase Inhibits IGF-I Signaling and Protein Synthesis in Vascular Smooth Muscle Cells via Stimulation of Insulin Receptor Substrate 1 S794

and Tuberous Sclerosis 2 S1345 Phosphorylation. Mol Endocrinol. 2010; 24:1218–29. [PubMed: 20363874]

250. Soares HP, Ni Y, Kisfalvi K, Sinnett-Smith J, Rozengurt E. Different Patterns of Akt and ERK Feedback Activation in Response to Rapamycin, Active-Site mTOR Inhibitors and Metformin in Pancreatic Cancer Cells. PLoS One. 2013; 8:e57289. [PubMed: 23437362]
251. Ming M, Sinnett-Smith J, Wang J, Soares HP, Young SH, Eibl G, et al. Dose-Dependent AMPK-Dependent and Independent Mechanisms of Berberine and Metformin Inhibition of mTORC1, ERK, DNA Synthesis and Proliferation in Pancreatic Cancer Cells. PLoS ONE. 2014; 9:e114573. [PubMed: 25493642]
252. Boukalova S, Stursa J, Werner L, Ezrova Z, Cerny J, Bezawork-Geleta A, et al. Mitochondrial Targeting of Metformin Enhances Its Activity against Pancreatic Cancer. Mol Cancer Ther. 2016; 15:2875. [PubMed: 27765848]
253. Kisfalvi K, Moro A, Sinnett-Smith J, Eibl G, Rozengurt E. Metformin Inhibits the Growth of Human Pancreatic Cancer Xenografts. Pancreas. 2013; 42:781–5. [PubMed: 23462329]
254. Mo J-S, Meng Z, Kim YC, Park HW, Hansen CG, Kim S, et al. Cellular energy stress induces AMPK-mediated regulation of YAP and the Hippo pathway. Nat Cell Biol. 2015; 17:500–10. [PubMed: 25751140]
255. Wang W, Xiao Z-D, Li X, Aziz KE, Gan B, Johnson RL, et al. AMPK modulates Hippo pathway activity to regulate energy homeostasis. Nat Cell Biol. 2015; 17:490–9. [PubMed: 25751139]
256. Omkumar RV, Darnay BG, Rodwell VW. Modulation of Syrian hamster 3-hydroxy-3-methylglutaryl-CoA reductase activity by phosphorylation. Role of serine 871. J Biol Chem. 1994; 269:6810–4. [PubMed: 8120043]
257. Rozengurt E, Soares HP, Sinnet-Smith J. Suppression of Feedback Loops Mediated by PI3K/mTOR Induces Multiple Overactivation of Compensatory Pathways: An Unintended Consequence Leading to Drug Resistance. Mol Cancer Ther. 2014; 13:2477–88. [PubMed: 25323681]
258. Tanti J-F, Jager J. Cellular mechanisms of insulin resistance: role of stress-regulated serine kinases and insulin receptor substrates (IRS) serine phosphorylation. Curr Opin Pharmacol. 2009; 9:753–62. [PubMed: 19683471]
259. Soares HP, Ming M, Mellon M, Young SH, Han L, Sinnet-Smith J, et al. Dual PI3K/mTOR Inhibitors Induce Rapid Overactivation of the MEK/ERK Pathway in Human Pancreatic Cancer Cells through Suppression of mTORC2. Mol Cancer Ther. 2015; 14:1014–23. [PubMed: 25673820]
260. Logsdon CD, Lu W. The Significance of Ras Activity in Pancreatic Cancer Initiation. Int J Biol Sci. 2016; 12:338–46. [PubMed: 26929740]
261. Swidnicka-Siergiejko AK, Gomez-Chou SB, Cruz-Monserrate Z, Deng D, Liu Y, Huang H, et al. Chronic inflammation initiates multiple forms of K-Ras-independent mouse pancreatic cancer in the absence of TP53. Oncogene. 2017; 36:3149–58. [PubMed: 27991926]

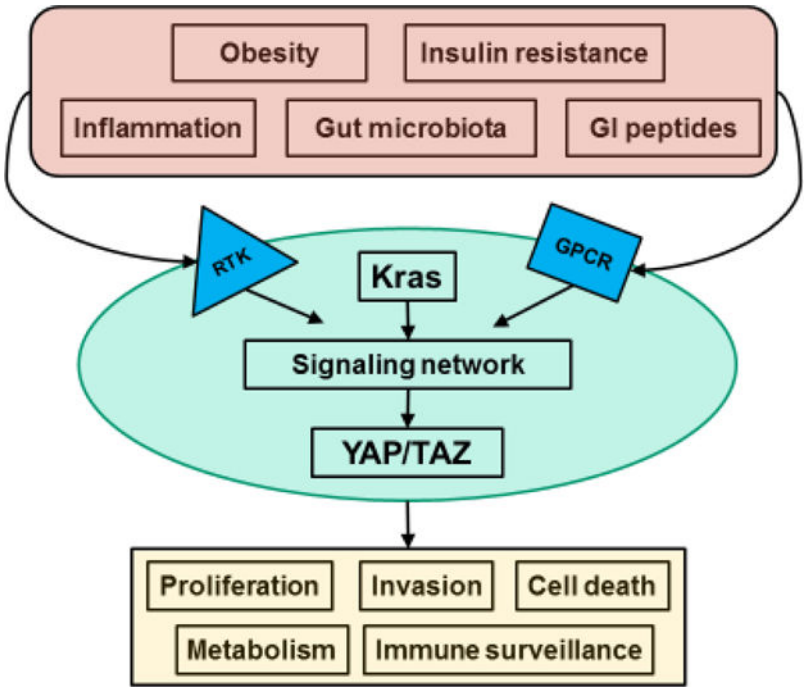


**Figure 1.**

Factors “upstream” of KRAS, e.g. obesity, insulin resistance, inflammation, gut microbiota, and GI peptides, reinforce and amplify the signaling network and feedback loops, including YAP/TAZ “downstream” of oncogenic KRAS, via receptor tyrosine kinase (RTK) and G protein-coupled receptors (GPCR) to fully and efficiently promote PDAC development and progression.

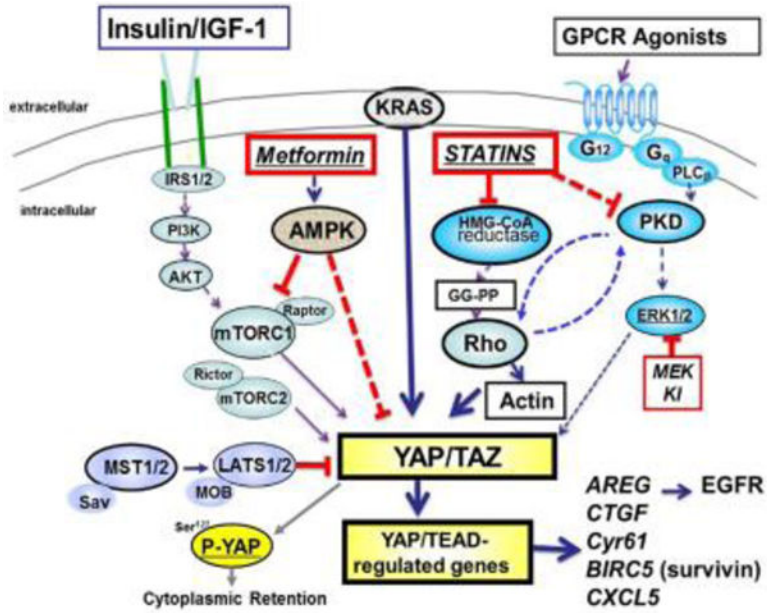


**Figure 2.**

Signaling network and feedback loops responsive to obesogenic signals and downstream of oncogenic Kras in PDAC with the transcriptional activators YAP/TAZ as converging and central regulators. FDA approved drugs metformin and statins disrupt this signaling network at different sites representing intriguing agents for PDAC prevention and interception.