Review

Repurposing of Metformin and Aspirin by Targeting AMPK-mTOR and Inflammation for Pancreatic Cancer Prevention and Treatment

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Abstract
Pancreatic cancer, as the fourth leading cause of cancer-related deaths, carries a poor prognosis with a median survival of 6 months and a dismal 5-year survival rate of 3% to 5%. These statistics highlight an urgent need for novel chemopreventive and therapeutic strategies for this malignancy. Metformin and aspirin have been explored as two emerging cancer chemoprevention agents for different types of cancers, including pancreatic cancer. Here, we review the effects of both metformin and aspirin on pancreatic tumorigenesis and their potential actions in pancreatic cancer. Special attention is paid to their effects on the important signaling pathways of pancreatic cancer development as well as possible mechanisms for synergy between these two agents. For metformin, the most important mechanism may involve the inhibition of mTOR signaling via AMP-activated protein kinase (AMPK)-dependent and -independent pathways. For aspirin, the major mechanism is the anti-inflammatory action through the inhibition of COX-1/COX-2 and modulation of the NFκB or STAT3 pathway. In addition, aspirin may activate AMPK, and both agents may affect Notch, Wnt/β-catenin, and other signaling pathways. The combination of metformin and aspirin will provide additive and possibly synergistic effects for the prevention and treatment of pancreatic cancer.

Introduction
An estimated 46,420 new cases of pancreatic cancer will be diagnosed in the United States in 2014, and 39,590 deaths will result from this disease, ranking pancreatic cancer as the fourth leading cause of cancer-related death (1). Despite efforts in the past 50 years, conventional treatment approaches, such as surgery, radiation, chemotherapy, or combinations of these, have had little impact on the course of this aggressive neoplasm, and the overall 5-year survival of all patients diagnosed with pancreatic cancer is still less than 5%. This poor survival highlights a critical need for novel chemoprevention and therapeutic strategies for prevention and treatment of this malignancy. Although several natural and synthetic agents are under development as potential chemoprevention agents for pancreatic cancer, there are no established recommendations for prevention of pancreatic cancer using pharmacologic agents (2).

In recent years, metformin and aspirin, two emerging candidates of cancer chemoprevention, have been explored for their clinical effects and underlying mechanisms in different types of cancers, including pancreatic cancer. In this review, we will first describe the epidemiologic results as well as in vitro (cell line systems) and in vivo (animal studies) evidence about metformin and aspirin for pancreatic cancer prevention. We will then discuss potential mechanisms of action of metformin and aspirin in pancreatic cancer by focusing on their effects on important cancer development signaling pathways and possible synergistic action of these two agents. Finally, we will conclude with a discussion of potential future research strategies for pancreatic cancer prevention and treatment using metformin and aspirin.

Inflammation and Pancreatic Tumorigenesis
Pancreatic tumorigenesis is generally thought to be associated with the accumulation of several genetic mutations, which lead to the activation of the oncogene Kras and inactivation of a number of tumor suppressor genes, including CDKN2A (also known as p16), SMAD4 (also known as DPC4), and TP53 (also known as p53; ref. 3). Several nongenetic risk factors including tobacco smoke, obesity, diabetes, and chronic pancreatitis have been identified to be associated with pancreatic cancer (4). Case-control studies have shown a positive association between smoking and pancreatic cancer with ORs of 1.3 to 5.53 (3). It is estimated...
that 25% to 30% of all pancreatic cancer–related deaths are attributed to tobacco use (6). Diabetes mellitus has been implicated both as an early manifestation of pancreatic cancer and as a predisposing factor. Meta-analysis and pooled analysis suggested that diabetes was associated with a 1.8 to 2.1-fold increase in risk of pancreatic cancer (7). In addition, pancreatic cancer has a known association with long-standing chronic pancreatitis and the rare condition of hereditary pancreatitis in humans (8). Patients with these two diseases are 17- and 53-times more likely to develop pancreatic ductal adenocarcinoma (PDAC) compared with unaffected controls, respectively (9).

Persistent low-grade inflammation is a characteristic of chronic pancreatitis and an important factor in the development of PDAC. In Kras-driven mouse models of PDAC, acute and chronic pancreatitis markedly accelerates pancreatic intraepithelial neoplasia (PanIN) and PDAC development (10, 11). These data illustrate the contribution of chronic inflammation to pancreatic carcinogenesis. Although it is not fully understood how inflammation enhances PDAC carcinogenesis, several mechanisms have been proposed and reviewed (12). For example, activation of oncogenic Kras creates an inflammatory tumor microenvironment by increasing the production of inflammatory mediators, including interleukin (IL)-6, IL-11, TNF-α, and IL-10. These inflammatory mediators activate STAT3 and/or NFκB in an autocrine or paracrine manner to promote cell survival and proliferation while maintaining an inflammatory tumor microenvironment (13). In addition, inflammation mediates suppression of immunosurveillance through inflammatory cytokine granulocyte-macrophage colony-stimulating factor (GM-CSF) produced by oncogenic Kras-expressing pancreatic neoplastic cells (12). Inflammation could also inhibit oncogene-induced senescence, stimulate the epithelial–mesenchymal transition, amplify and prolong Ras activity, and promote oncogenic mutagenesis, all of which contribute to PDAC initiation, development, and metastasis (14). Taken together, these observations support the importance of pancreatic inflammation, mediated by cytokines, reactive oxygen species, and unregulated proinflammatory pathways, for the development and progression of human pancreatic malignancy.

The Effects of Metformin on Pancreatic Tumorigenesis

Clinical evidence

Metformin (1,1-dimethylbiguanide hydrochloride), a biguanide derivative, is the most widely prescribed drug to treat hyperglycemia in individuals with type II diabetes. Recently, metformin use has been associated with a decreased risk of specific cancers, including prostate, colon, liver, pancreas, and breast cancers (15). Several epidemiologic studies have linked the administration of metformin with a reduced risk of pancreatic cancer in patients with type II diabetes mellitus. Li and colleagues (16), for example, reported that use of metformin was associated with a 62% lower risk of developing pancreatic cancer compared with metformin nonuse [OR, 0.38; 95% confidence interval (CI), 0.22–0.69; P = 0.001]. In a Taiwanese cohort of 480,984 participants, metformin was reported to significantly reduce the incidence of pancreatic cancer in diabetic patients (OR, 0.15; 95% CI, 0.03–0.79; ref. 17). Recently, another group reported a decreased risk of pancreatic cancer with metformin in women only (OR, 0.43; 95% CI, 0.23–0.80; ref. 18). Furthermore, a retrospective study of diabetic patients with pancreatic cancer revealed an improved survival for patients using metformin as diabetes treatment (OR, 0.68; 95% CI, 0.52–0.89; P = 0.004; ref. 19). However, a meta-analysis of nine observational studies (6 cohort and 3 case–control) showed a trend but not a significant association between metformin and the risk of developing pancreatic cancer (adjusted OR, 0.76; 95% CI, 0.57–1.03; P = 0.073; ref. 20), indicating a significant heterogeneity in clinical studies on the relationship between metformin use and pancreatic cancer risk.

Experimental data

Several in vitro studies have established a direct effect of metformin on many types of cancer cells, including those of pancreatic cancer (21, 22). Many cellular studies have focused on AMP-activated protein kinase (AMPK) and related molecules and have revealed an anticancer action of metformin in vitro. In animal studies, metformin has been shown to prevent the promotional effect of high-fat diet on N-nitrosobis(2-oxopropyl)amine (BOP)-induced pancreatic carcinogenesis in Syrian hamsters (23) and inhibit the growth of pancreatic cancer cells (MIA PaCa-2 and PANC1) in xenograft models in athymic nude mice (21). Collectively, these preclinical data provided biologically plausible evidence that metformin could be used for the prevention and/or treatment of pancreatic cancer.

The Effects of Aspirin on Pancreatic Tumorigenesis

Clinical evidence

Aspirin and other nonsteroidal anti-inflammatory drugs (NSAID) show promise as cancer chemoprevention agents due to their anti-inflammatory properties (24). However, findings from epidemiologic studies of aspirin and NSAID use in relation to pancreatic cancer risk have been inconsistent. Six studies reported that aspirin and non-aspirin NSAID use are not associated with pancreatic cancer risk, whereas two studies reported an increased risk of pancreatic cancer (25). In contrast, two other studies reported that aspirin or non-aspirin NSAID use is associated with a decreased risk of pancreatic cancer (25). Using systematic meta-analyses, two studies summarized the available epidemiologic evidence on the relationship between aspirin or non-aspirin NSAID exposure and risk of pancreatic cancer, and both studies indicated null associations (26, 27). In a pooled analysis of 25,570 patients in eight trials, Rothwell and colleagues recently reported that daily aspirin use reduced deaths of several common cancers, including significant reductions in colorectal and pancreatic cancer...
deaths, with most benefit seen after 5 years of the scheduled treatment (28). In a clinic-based case–control study, we showed that aspirin use, but not non-aspirin NSAID use, is associated with lowered risk of developing pancreatic cancer (25).

**Experimental data**

Laboratory studies have shown that aspirin use inhibits pancreatic tumor formation in orthotopic mouse models (29, 30) and genetically engineered mouse models (31, 32). Scabas and colleagues (29) observed that animals given aspirin for 6 days before tumor cell injection had a lower incidence of tumor formation compared with those receiving aspirin 2 weeks after injection. They suggested that aspirin-mediated inhibition of NFκB activation in inflammation is a possible mechanism for the cancer preventive effect of aspirin. Support for this hypothesis is provided by experiments performed by Fendrich and colleagues (31) and Rao and colleagues (32) in which aspirin and low-dose nitric oxide–releasing aspirin delay progression of PanINs and even the incidence of tumor formation in a genetically engineered mouse model of pancreatic cancer. These data indicate that aspirin-mediated anti-inflammation approaches could be an effective strategy to prevent pancreatic carcinoma. Aspirin may influence several molecular pathways. However, the underlying molecular mechanism remains enigmatic.

**Pharmacodynamic and Pharmacokinetic for Metformin and Aspirin**

**Metformin**

Metformin is always administered orally in clinical practice, and its oral bioavailability is about 50% to 60% under fasting conditions. Because of its low lipophilicity, metformin does not passively diffuse through cell membranes and must be actively transported into cells by the transmembrane protein organic cation transporter 1 and 2, which are highly expressed in hepatic cells and primary PDAC cells (33). Once absorbed, metformin rapidly accumulates in the liver, stomach, duodenum, salivary glands, and kidneys. No metabolites or conjugates of metformin have been identified and the drug is mainly excreted unchanged via the kidneys. After a single dose, peak plasma concentrations of metformin in patients with diabetes are in the range of 0.5 to 2 μg/mL (3.02 to 12.08 μmol/L) within 1 to 3 hours of taking immediate-release metformin and 4 to 8 hours with extended-release formulations. Therefore, the current preclinical data should be interpreted with caution, because most in vitro studies in the literature use supra-physiologic concentrations of metformin (mmol/L range) to observe modulation of signaling pathways or growth inhibition.

**Aspirin**

Aspirin (acetylsalicylic acid) is a weak organic acid, and is absorbed in the stomach and the upper small intestine with oral bioavailability of 68%. Aspirin is rapidly transformed into the active metabolite, salicylate in the stomach, intestinal mucosa, blood, and liver, and liver is the main site of biotransformation. About 50% to 80% of salicylate in the blood is bound to albumin protein in a concentration-dependent manner, while the rest remains in the active, ionized state. Plasma salicylate levels in general range from 30 to 100 mg/L (0.19–0.63 mmol/L) after usual therapeutic doses, 50 to 300 mg/L (0.31–1.87 mmol/L) in patients taking high dose and 700 to 1,400 mg/L (4.37–8.75 mmol/L) following acute overdose. Most preclinical experiments used aspirin <5 mmol/L (cell line systems) and <50 mg/kg (animal studies), which are the physiologically achievable concentrations in humans. Salicylate is primarily metabolized by hepatic conjugation with glycone to form salicylic acid or with glucuronic acid to form salicyl acyl and phenolic glucuronide. Small amounts of salicylic acid are also hydroxylated to gentisic acid. Salicylates are excreted mainly by the kidneys as salicylic acid (75%), free salicylic acid (10%), salicylic phenol (10%), and acyl glucuronides (5%), gentisic acid (<1%), and 2,3-dihydroxybenzoic acid (34).

**Mechanisms by Which Metformin and Aspirin Inhibit mTOR and Inflammation**

**Inhibition of mTOR signaling pathway**

mTOR (the mammalian target of rapamycin), a 289 kDa serine/threonine kinase, is a downstream effector of the phosphatidylinositol 3-kinase (PI3K)/AKT pathway. The PI3K/AKT/mTOR pathway is activated downstream of RAS signaling and likely represents a major mediator of RAS-driven oncogenesis (35). In human pancreatic cancer, the PI3K/Akt/mTOR pathway is deregulated in the majority of tumors, and the activation of this pathway correlates significantly with a poor prognosis (35). It has been reported that mTOR complex 1 (mTORC1) signaling plays a pivotal role in the proliferation and survival of pancreatic cancer cells and is activated in pancreatic cancer tissues (36, 37). Recently, the mTOR pathway has been shown to play critical roles in pancreatic cancer stem cells through specific and stemness-related functions (35). Both metformin and aspirin may abolish mTOR activation through an AMPK-dependent or -independent mechanism.

**AMPK-dependent mechanisms.** The AMPK-dependent antitumoral actions of metformin originate from liver kinase B1 (LKB1)–mediated AMPK activation and subsequent suppression of the downstream mTOR signaling pathways (Fig. 1; ref. 15). Metformin can inhibit mitochondrial respiratory chain complex I, which reduces ATP production, thus leading to the activation of AMPK (38). AMPK is a highly conserved protein kinase that exists in all eukaryotic cells. The main upstream kinase that phosphorylates AMPK is LKB1, which has been found mutated in many different cancers (39). Metformin-mediated AMPK activation leads to an inhibition of mTORC1, a reduction in phosphorylation of its major downstream effectors, the eukaryotic initiation factor 4E-binding proteins (4E-BP) and ribosomal protein S6 kinases (S6Ks), in a tuberous sclerosis complex 1/2-dependent manner (15, 40).
Salicylate (>1 mmol/L), as a main metabolite of aspirin, was recently reported to directly activate AMPK activity in human embryonic kidney (HEK) 293 cells and mice in vivo (41). Din and colleagues investigated the effects of aspirin on AMPK/mTOR signaling and showed that aspirin alone or the combination of aspirin and metformin activates AMPK and inhibits mTOR signaling in colorectal cancer cells (42), suggesting that AMPK–mTOR are also direct targets of aspirin. However, recent evidence suggests that metformin and aspirin may exert their anticancer effects through pathways independent of AMPK activation.

**AMPK-independent mechanisms.** Metformin may diminish the effects of insulin on tumor development and growth through inhibition of hepatic gluconeogenesis or increased insulin sensitivity, thus leading to reduced circulating insulin and insulin-like growth factor (IGF)-1 levels (Fig. 1). It is generally accepted that metformin-induced activation of AMPK is required for inhibition of hepatic gluconeogenesis; however, Foretz and colleagues suggested that metformin-induced inhibition of glucose production is mediated directly by the elevated AMP/ATP ratio in the liver (43). Miller and colleagues also showed that metformin-induced activation of AMPK was not required for decreasing the expression of gluconeogenic genes and glucose production (44). This group recently demonstrated that metformin may inhibit hepatic gluconeogenesis, independently of AMPK, through reducing cAMP levels, inhibiting of protein kinase A activity and blocking glucagon-dependent glucose production (45).

Insulin and IGF-1 are key factors in promoting cancer development. Binding of insulin/IGF-1 to their receptors results in receptor autophosphorylation and activation of receptor tyrosine kinase, followed by tyrosine phosphorylation of insulin receptor substrates (IRS 1–4), which further propagates the downstream PI3K/AKT/mTOR signaling pathway (46). Metformin may abolish mTOR activation through inhibition of insulin/IGF-1 signaling. Metformin was reported to reduce circulating insulin levels by 22% and improve insulin sensitivity by 25% in non-diabetic women with breast cancer (47). Accordingly, metformin suppressed the promotional effects of high-fat diet-induced peripheral insulin resistance on pancreatic tumorigenesis by improving insulin sensitivity and lowering circulating insulin (23, 48), suggesting the insulin-lowering effects of metformin as a potential mechanism of action in the prevention and treatment of pancreatic cancer. Interestingly, high doses of salicylates were also reported to decrease insulin signaling by improving insulin sensitivity through the inhibition of phosphorylation of IRS1 and degradation of 1KB kinase α and β (49, 50). Overall, the insulin-lowering effects of metformin and aspirin may play a pivotal role in their anticancer activity as insulin has mitogenic and pro-survival effects and tumor cells often express high levels of the insulin receptor, indicating a
potential sensitivity to the growth-promoting effects of the hormone (51).

In addition, under particular conditions metformin can directly suppress mTORC1 through inhibition of the Rag GTPase (32) or through induction of REDD1 (regulated in development and DNA damage responses 1; ref. 53). It has been shown that metformin acts through p53 to increase REDD1 expression, independently of AMPK, and this ultimately leads to mTOR inhibition and cell-cycle arrest (53). Similarly, aspirin is also shown to inhibit mTOR pathway through AMPK-independent mechanisms (42). Nevertheless, there is a lack of complete understanding of the precise molecular mechanism of action of metformin and aspirin on pancreatic tumorigenesis. Further research to elucidate the mechanism by which metformin and aspirin activate AMPK and inhibit mTOR would be beneficial to understanding the prevention of pancreatic cancer or other human cancers.

**NFκB/STAT3 signaling pathway**

NFκB, a major transcription factor for inflammatory responses, plays a significant role in carcinogenesis and is now emerging as a link between inflammation and cancer (54). NFκB is constitutively activated in 70% of human pancreatic cancer and in many human pancreatic cancer cell lines, but not in normal pancreatic tissues or in immortalized, nontumorigenic pancreatic epithelial cells (55, 56). It has been shown that NFκB signaling is activated by oncogenic Kras or by cytokines secreted from malignant or inflammatory genes, such as COX-2 (63). The dysregulation of these genes due to the activation of cytokine receptors. STAT3 also interacts directly with p65 (RelA), a NFκB family member, and facilitates its nuclear translocation. Activation of COX-2 and STAT3 coregulate numerous oncogenic and inflammatory signaling components, such as COX-2, COX-1 (64), which activate STAT3 in an autocrine manner (60). Moreover, inhibition of constitutive NFκB activity by a phosphoallylation-defective IκBζ (S32, 36A; IκBζ-M) suppresses pancreatic tumorigenesis in an orthotopic nude mouse model (57).

STAT3 mediates a complex spectrum of cellular response including inflammation, cell proliferation, and apoptosis. STAT3 integrates signals from cytokines and growth factors into transcriptional responses in target cells and may serve as a mediator of inflammation-associated processes, such as pancreatitis-driven PanIN development. Constitutive activation of STAT3 has been reported in 30% to 100% of human PDAC tumor specimens, as well as in many PDAC cell lines (58, 59). It has also been shown that Kras activation activates PanIN to produce cytokines such as IL-6 and IL-11, which activate STAT3 in an autocrine manner (60). Moreover, loss of STAT3 reduces acinar-to-ductal metaplasia and PanIN formation induced by oncogenic Kras (60, 61). Interestingly, cross-talk between the NFκB and the STAT3 pathways has been suggested through the release of IL-6 and other cytokines and the autocrine/paracrine activation of cytokine receptors. STAT3 also interacts directly with p65 (RelA), a NFκB family member, and facilitates its acetylation and transcriptional activity in tumors (62). In fact, NFκB and STAT3 coregulate numerous oncogenic and inflammatory genes, such as Myc, Bcl-xl, c-myc, cyclin D1, COX-2, and IL-1β (63). The dysregulation of these genes due to the persistent activation of both NFκB and STAT3 in tumors and tumor microenvironment is crucial for the progression of tumor. These studies suggest that the NFκB/STAT3 signaling pathway plays a critical role in inflammation-mediated pancreatic carcinogenesis.

Aspirin irreversibly inhibits COX-1 and modifies the enzymatic activity of COX-2 (Fig. 1). COX-1 is expressed constitutively in a variety of tissues and plays an important role in homeostatic processes (64). COX-2, an inducible enzyme and downstream of NFκB and STAT3, produces predominately proinflammatory prostaglandins (65). Furthermore, COX-2 is expressed in 57% of human pancreatic carcinoma specimens (66), and its mRNA levels were increased more than 60-fold in patients with pancreatic cancer compared with normal controls (67), suggesting a key role for COX-2 in pancreatic cancer. In addition, aspirin has been shown to inhibit NFκB activation through specific inhibition of IKK-2 activity by binding to IKK-2 and reducing ATP binding (68, 69). Aspirin has also been reported to induce apoptosis through down-regulation of the IL-6-STAT3 signaling pathway in human glioblastoma cells (70) and in a murine model of colorectal cancer (71). However, the potential involvement of NFκB and STAT3 in the anticancer effects of the combination of metformin and aspirin on pancreatic cancer is not clearly understood.

Although AMPK-dependent suppression of mTOR signaling remains the key mechanism of the antitumor action of metformin, metformin may also target the inflammatory component present in the microenvironment of most neoplastic tissues, leading to tumor reduction. Metformin has been reported to inhibit IκB kinase phosphorylation, IκBα degradation, and IL-6 production through AMPK activation (72–74). A previous study suggested that metformin inhibited STAT3 phosphorylation and downstream signaling in triple-negative breast cancer cells, suggesting that STAT3 is a critical regulator of metformin action (75). Recently, metformin has also been shown to increase the sensitivity of resistant cells to cisplatin by suppressing STAT3 activity without activation of AMPK (76). It has been suggested that AMPK suppresses NFκB signaling indirectly via its downstream mediators, such as sirtuin 1, forkhead box protein O (FoxO) family, peroxisome proliferative activated receptor gamma coactivator 1α, and p53; these factors can subsequently repress the expression of inflammatory factors (77). However, whether inhibition of NFκB and STAT3 by metformin in pancreatic cancer cells requires AMPK activation needs further investigation.

**Additional Signaling Pathways Targeted by Metformin and Aspirin**

**Notch signaling pathway**

The Notch signaling pathway is a highly evolutionarily conserved pathway that mediates cell-to-cell communication. The well-documented functions regulated by Notch signaling include the maintenance of stem cell populations, determination of cell fate, and the regulation of proliferation and apoptosis (78). Notch signaling in pancreatic cancer has been extensively studied and recently reviewed...
by Avila and Kissil (79). Notch pathway components are highly expressed in pancreatic adenocarcinoma, but the role of Notch signaling in pancreatic cancer remains unresolved. Coactivation of Kras<sup>G12D</sup> and the Notch1-intracellular domain in mature acinar cells led to a significantly higher number of PanIN lesions compared with activation of Kras<sup>G12D</sup> alone (80). Pdx1-Cre; LSL-Kras<sup>G12D</sup>; p53<sup>lox/lox</sup> mice treated with a γ-secretase inhibitor, which inhibits Notch signaling inhibitor, do not develop PDAC (81). These data support an oncogenic role for Notch signaling in pancreatic tumorigenesis. In contrast, other groups demonstrated that Notch 1 suppresses PanIN formation, and deletions of both Notch1 alleles caused a slight decrease in the median survival of PDAC-bearing mice (82, 83). It is possible that Notch may function as a tumor suppressor to inhibit PanIN development at an early stage but act as an oncogene to promote pancreatic cancer progression at later stages.

Interestingly, both NFκB and Notch pathways are activated in many types of cancer, including pancreatic cancer (12). As described above, NFκB signaling may be activated by oncogenic Kras. The activation of NFκB can activate Notch signaling, and Notch signaling synergizes with Kras to accelerate PDAC development (84). In addition, activated Notch signaling suppresses the anti-inflammatory transcription factor peroxisome proliferator-activated receptor-γ, leading to constitutive production of inflammatory mediators by malignant cells in PDAC (84). Therefore, Notch seems to be of significance in pancreatic carcinogenesis, and cross-talks among Kras, NFκB, Notch, and COX-2 in cellular signaling might contribute to the molecular pathogenesis of pancreatic cancer. One recent study reported that metformin downregulated the mRNA expression of Notch1 in pancreatic cancer cells (85). Additional studies are needed to determine whether metformin and aspirin affect Notch signaling pathways during inflammation and pancreatic carcinogenesis.

### Wnt/β-catenin Pathway

Wnt signaling is involved in normal embryonic development and homeostatic tissue self-renewal, and the canonical Wnt/β-catenin pathway has been implicated in a variety of cancers including liver, colorectal, breast, prostate, renal, and pancreatic cancers (86, 87). Wnt signaling regulates numerous aspects of pancreatic biology, and its activity is gradually increased during pancreatic carcinogenesis. The accumulation of β-catenin and activation of Wnt target genes have been observed in PanINs and PDAC (87, 88). Inhibition of Wnt signaling has been shown to reduce proliferation and increase apoptosis of pancreatic cancer cells in vitro. In addition, β-catenin functionally supports maintenance of PDAC cell proliferation and tumor-forming capacity in xenograft models (89, 90). Recently, Zhang and colleagues showed that inhibition of Wnt signaling significantly delayed PanIN formation in an established transgenic mouse model of pancreatic cancer (91). These data indicate that activation of the Wnt/β-catenin pathway is required and critical for the initiation and progression of pancreatic cancer.

Aspirin and metformin have been reported to modulate the Wnt/β-catenin signaling. Aspirin downregulates the constitutively active Wnt/β-catenin signaling in colorectal cancer cells by increasing the phosphorylation of β-catenin (92). Aspirin is also reported to induce the mitochondria/caspase-3 apoptotic pathway, which is dependent on the Wnt/β-catenin signaling in mesenchymal stem cells (93). For metformin, it has been reported to inhibit the activation of Wnt/β-catenin signaling in cervical cancer cells (94). Metformin has also been reported to increase the expression of Bambi, a TGF-β decoy receptor, and induce prosurvival Wnt/β-catenin signaling in hepatic stellate cells (95). To date, the effect of metformin and aspirin on the Wnt/β-catenin pathway has not been evaluated in pancreatic cancer cells. Given that the Wnt/β-catenin pathway plays an important role in pancreatic tumor development, more research is needed to elucidate the effect of metformin and aspirin on this signaling pathway in pancreatic cancer.

### G protein–coupled receptor signaling

In addition to their effects on AMPK/mTOR, NFκB/STAT3, Notch, and Wnt/β-catenin signaling pathways, metformin and aspirin have also been shown to be involved in other signaling pathways. Metformin can disrupt cross-talk between G protein–coupled receptor (GPCR) and insulin receptor signaling systems in pancreatic cancer cells (21). Low doses of metformin prevent insulin-induced augmentation of Ca<sup>2+</sup> signaling, DNA synthesis, and the anchorage-dependent and anchorage-independent proliferation in response to stimulation with GPCR agonists (e.g., neurotensin, bradykinin, and angiotensin II) through an AMPK-dependent way (21).

### Cancer stem cells

Cancer stem-like cells (CSC), which have self-renewal and multi-lineage differentiation capacities, are becoming a new target for cancer drug discovery. Metformin can selectively kill CSCs in several different cancers, including pancreatic cancer (85, 96, 97). The alteration of microRNA expression profiles (85) and the activation of FoxO3 by metformin (98) may be involved in this inhibitory effect. Given that the inflammatory pathway is necessary for the transformation from normal cells to cancer cells (99), metformin can also block the transformation and cancer stem cell formation via the specific inhibition of the NFκB pathway in breast cancer stem cells (96). Whether aspirin has any selectivity to kill CSCs and decrease the expression of CSC markers has so far been unexplored.

### Evidence for Synergy between Metformin and Aspirin against Pancreatic Cancer

Combination chemoprevention has been considered as a promising effective strategy for enhanced cancer chemopreventive action (2, 100). It is possible that a combination of metformin and aspirin may synergistically activate AMPK and inhibit the mTOR and NFκB/STAT3 pathways, which could increase the efficacy for the prevention of pancreatic cancer and other cancers. Din and colleagues recently
showed that the combination of aspirin and metformin has an additive effect on AMPK activation and mTOR inhibition in colorectal cancer cells (42). Furthermore, they observed that aspirin increases the ADP:ATP ratio, a surrogate for AMP:ATP ratio.

In our laboratory, we observed that the combination of metformin and aspirin at low concentrations (1–5 mmol/L) had significant synergistic effects on the inhibition of cell viability, migration, and colony formation in pancreatic cancer cell lines PANC-1 and BxPC-3 (unpublished data). The combination of metformin and aspirin also significantly inhibited the phosphorylation of mTOR, S6K, JAK2, and STAT3, as well as the protein and mRNA expression levels of BCL-2 and MCL-1, which are the two downstream targets of STAT3. Furthermore, we also observed increased apoptosis as measured by caspase-3 and PARP cleavage in PANC-1 and BxPC-3 cells treated with the combination of metformin and aspirin (unpublished data). Our results suggest that metformin and aspirin synergistically induce cell death in pancreatic cancer cells associated with downregulating Bcl-2 and Mcl-1 through the STAT3 pathway. These results support the hypothesis that the combination of metformin and aspirin can be effective chemopreventive agents against pancreatic cancer. In future studies, it is a challenge to elucidate the specific targets or pathways that are affected by these two agents, as well as the mechanism by which the synergy is generated.

Conclusions and Perspectives

The poor response of pancreatic cancer to therapy (101) provides an important reason for finding effective chemopreventive and therapeutic agents for pancreatic cancer. There are several natural, diet-derived bioactive compounds that have been evaluated as pancreatic cancer chemopreventive agents. Among them, curcumin (diferuloylmethane), a bioactive component of the spice turmeric extracted from the rhizomes of the plant Curcuma longa, has been extensively studied and is known to have numerous biologic activities (102). It has been demonstrated that curcumin inhibits the proliferation of pancreatic cancer cells and sensitizes them to gemcitabine, celecoxib, and paclitaxel through inhibiting NFκB-regulated gene products, inducing apoptosis and suppressing angiogenesis (2). However, because of its poor absorption and low bioactivity, it is extremely challenging to develop curcumin as a chemoprevention agent against pancreatic cancer. For any chemoprevention agents, an important consideration is the balance between efficacy and the side effects of long-term use of the agent. Long-term use of metformin is associated with few adverse effects, while many epidemiologic studies revealed a strong correlation between metformin and a lower risk of numerous cancers (103, 104). The use of aspirin needs to be balanced against an increased risk of bleeding in some individuals, but it is currently one of the most attractive candidates for the chemoprevention of colorectal cancer (105).

To reduce the dosages of metformin and aspirin, and avoid possible gastrointestinal irritation caused by aspirin, metformin and aspirin may be incorporated into solid-lipid nanoparticles. On theory, these nanoparticles can be prepared with stearic acid using a hot melt, oil-in-water emulsion technique; the resulting nanoparticles consist of a solid-lipid core and are stabilized by surfactants (106, 107). Aspirin and metformin may form an acid–base complex and be encapsulated in the nanoparticles at high efficiency. Similar types of nanoparticles have been reported to be stable and suitable for oral delivery (108). They are absorbed through the lymphatic system and then enter the systemic circulation, hence passing gastrointestinal metabolism (109, 110). A recent study by Grandhi and colleagues (111) has demonstrated high efficacy of the combination of aspirin and curcumin in such nanoparticles in the prevention of pancreatic cancer in an animal model. The toxicity profile of the combination of metformin and aspirin as well as the nanoparticle delivery systems needs to be systematically studied.

Six phase II clinical trials combining metformin and other chemotherapeutic drugs, and a phase I clinical trial on metformin pharmacodynamics in patients with pancreatic cancer are ongoing or upcoming. However, there are currently no clinical trials for chemoprevention effect of either metformin or aspirin in pancreatic cancer. In those phase II clinical trials, the investigators mainly focus on the median survival and the response rate of patients, as well as the toxicity. In the phase I clinical trial (NCI01954732), the cell proliferation using Ki67 as a biomarker and the phosphorylation of mTOR will be determined in pancreatic tumor tissues. These results will give a direct evaluation of the biologic effects of metformin in pancreatic cancer patients, which will be a new perspective and an important implication of future clinical studies.

As noted above, there are still largely unknown mechanisms underlying many of the actions of metformin and aspirin for the prevention of pancreatic cancer. Although AMPK is strongly related to the protective effects of metformin against the development of cancer, AMPK is not involved in all pathways affected by metformin. Therefore, a comprehensive exploration of the mechanisms of metformin and aspirin in pancreatic cancer is necessary and urgent for the launch of clinical trials in the future. In addition, the relationship between diabetes and pancreatic cancer is complex and intertwined. An improved understanding of the relationship among diabetes, metformin, and the development of pancreatic cancer would provide new research avenues for developing metformin as a chemopreventive or adjuvant therapeutic agent for pancreatic cancer. The activity and molecular mechanisms of metformin action in nondiabetic pancreatic cancer has not yet been fully determined. Further studies on the chemopreventive value of metformin in nondiabetic patients are warranted. In view of anti-inflammatory property of aspirin and the increased risk of PDAC in patients with pancreatitis, patients with pancreatitis may benefit most from taking metformin and aspirin. In addition, obesity, which is associated with elevations in insulin/IGF-1 (112) and increases of low-grade chronic inflammation (113), confers a higher risk of PDAC. Metformin and aspirin, having both insulin-lowering and...
anti-inflammatory effects, may be of particular value in obesity.

It is noteworthy that inflammation is essential to the tumorigenic microenvironment by providing growth factors, survival factors, proangiogenic factors, and other inductive signals (114). NFκB and STAT3 are two important transcription factors involved in inflammation by integrating cytokine-induced signaling pathways (54), and metformin and aspirin are both inhibitors of NFκB and STAT3, suggesting a mechanism of anti-inflammation and cancer prevention by these agents. Considering the importance of the AMPK-mTOR pathway and inflammation in pancreatic cancer development, integrated studies on the mechanisms of synergistic action of metformin and aspirin would provide the scientific basis for the development of a new strategy for prevention and treatment of pancreatic cancer.

**References**


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