Published OnlineFirst April 13, 2010; DOI: 10.1158/1078-0432.CCR-09-2229

Molecular Pathways

Clinical Cancer Research

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

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Abstract

Insulin/insulin-like growth factor 1(IGF-1) receptors and G protein-coupled receptors (GPCR) signaling systems are implicated in autocrine-paracrine stimulation of a variety of malignancies, including ductal adenocarcinoma of the pancreas, one of the most lethal human diseases. Novel targets for pancreatic cancer therapy are urgently needed. We identified a crosstalk between insulin/IGF-1 receptors and GPCR signaling systems in pancreatic cancer cells, leading to enhanced signaling, DNA synthesis, and proliferation. Crosstalk between these signaling systems depends on mammalian target of rapamycin (mTOR) complex 1 (mTORC1). Metformin, the most widely used drug in the treatment of type 2 diabetes, activates AMP kinase (AMPK), which negatively regulates mTORC1. Recent results show that metformin-induced activation of AMPK disrupts crosstalk between insulin/IGF-1 receptor and GPCR signaling in pancreatic cancer cells and inhibits the growth of these cells in xenograft models. Given that insulin/IGF-1 and GPCRs are implicated in other malignancies, a similar crosstalk mechanism may be operative in other cancer cell types. Recent epidemiological studies linked administration of metformin with a reduced risk of pancreatic, breast, and prostate cancer in diabetic patients. We posit that crosstalk between insulin/IGF-1 receptor and GPCR signaling is a mechanism for promoting the development of certain types of cancer and a target for the prevention and therapy of these diseases via metformin administration. *Clin Cancer Res; 16(9); 2505–11. ©2010 AACR.*

Background

Crosstalk between the insulin/insulin-like growth factor 1 receptor and G protein-coupled receptor signaling systems in pancreatic cancer cells. The major challenge facing cancer research is to identify novel molecules and develop strategies for the treatment of common solid tumors, including ductal adenocarcinoma of the pancreas, a devastating disease with overall 5-year survival rate of only 3 to 5%. Thus far, a range of targeted therapies against epidermal growth factor receptor (EGFR), RAS/MEK, and vascular endothelial growth factor (VEGF) have failed to improve survival significantly in clinical trials. Novel targets for therapeutic intervention are urgently needed and will most likely arise from a more detailed understanding of the signaling pathways that stimulate the unrestrained proliferation and invasiveness of these cancer cells.

Signaling pathways do not act in isolation, but crosstalk (i.e., interact) with each other, forming complex signaling networks (1). Multiple lines of evidence support the notion that crosstalk between the insulin/insulin-like growth factor 1 (IGF-1) receptor and heptahelical G protein-coupled receptor (GPCR) signaling systems plays a critical role in the regulation of multiple physiological functions and in a variety of abnormal processes, including cardiovascular and renal pathologies in obesity, metabolic syndrome, and type II diabetes (see ref. 2 for references). In turn, epidemiological studies linked long standing type 2 diabetes, obesity, and metabolic syndrome with *increased* risk for developing cancer, including pancreatic and colon cancer (discussed below). At the cellular level, insulin dramatically synergizes with GPCR agonists in inducing mitogenic signaling (3), and many GPCRs and their cognate agonists are implicated as autocrine-paracrine growth factors for multiple solid tumors, including pancreas, colon, prostate, and breast (1, 4). The purpose of this article is to discuss recent advances in identifying crosstalk between insulin/IGF-1 receptor and GPCR signaling pathways as a mechanism for enhancing pancreatic cancer cell proliferation and a target for the antidiabetic agent metformin, a potential new tool in the prevention and/or therapy of this disease.

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doi: 10.1158/1078-0432.CCR-09-2229

Published OnlineFirst April 13, 2010; DOI: 10.1158/1078-0432.CCR-09-2229

GPCR signaling. Human pancreatic adenocarcinoma cells express multiple GPCRs that mediate rapid signaling and cell proliferation in response to their corresponding agonists (2, 5–8) and expression of angiotensin (ANG II), and neurotensin GPCRs are markedly increased in pancreatic cancer tissues (9–12). Neurotensin acts as a potent growth factor for pancreatic cancer cells (5, 7, 8, 13), and a broad-spectrum GPCR antagonist inhibited the growth of pancreatic cancer cells either *in vitro* or xenografted into nu/nu mice (14). Transgenic expression of the gastrin GPCR in murine pancreas induced neoplastic proliferation and acinar-ductal transdifferentiation (15).

Heterotrimeric guanine nucleotide-binding regulatory proteins (G proteins), composed of α, β, and γ subunits, transduce external signals from heptahelical receptors to intracellular effectors. G proteins are classified according to their α subunits into four subfamilies: Gs, Gi, Gq, and G_{12}. Agonist binding to GPCRs induces movement of the transmembrane-spanning segments and promotes a conformational change in the cytoplasmic domain of the receptor that leads to the exchange of GDP bound to the α subunit of the G protein for GTP. The resulting GTP-Gα and Gβγ subunits stimulate effectors that transduce GPCR activation into multiple downstream signaling responses. These include a rapid increase in the activity of phospholipases C (PLC), D, and A_2, leading to the synthesis of lipid-derived second messengers, Ca^{2+} fluxes, elevation of cAMP, and subsequent activation of serine and tyrosine protein phosphorylation cascades, including protein kinase C/protein kinase D (PKC/PKD), Raf/MEK/ERK, mammalian target of rapamycin (mTOR)/p70S6K, and Src/FAK. GPCR signaling leading to mitogenesis has been recently reviewed elsewhere (1), and here we focus on studies particularly relevant to pancreatic cancer cells. Some of the major pathways shown in these cells are shown in Fig. 1. Specifically, GPCR-induced Gαq/PLC activation stimulates the hydrolysis of phosphatidylinositol 4, 5-bisphosphate (PIP_2) to produce two second messengers: inositol 1,4,5-trisphosphate (IP_3) and 1,2-diacylglycerol (DAG). IP_3 triggers the release of Ca^{2+} from internal stores, leading to a rapid and transient increase in intracellular Ca^{2+} concentration. DAG remains in the plane of the membrane and directly activates PKC, a phospholipid-dependent protein kinase family. PKCs induce rapid phosphorylation of cytosolic and membrane-bound proteins, including the downstream protein kinases of the PKD family (16). PKD induces MEK/ERK/p90RSK pathway activation and thereby mediates cell proliferation in some cell types (17, 18). PKD is activated in pancreatic cancer cells by GPCR agonists, including neurotensin (6, 19), it is overexpressed in pancreatic cancer tissues (20), and its overexpression promotes pancreatic cancer cell proliferation (21). The PKD family is emerging as an important downstream element in GPCR-induced mitogenic signaling in pancreatic cancer.

GPCR agonists also stimulate mTOR-dependent pathways in many model systems, including pancreatic cancer cells, but the molecular pathways remain incompletely understood (1). mTORC1 is likely to be activated in response to GPCR agonists through the ERK/RSK pathway rather than via phosphatidylinositol 3-kinase (PI3K)/Akt, which is activated weakly by GPCR agonists in pancreatic cancer cells (22). As further developed below, mTORC1 activation is a site of signal convergence and dissemination in the crosstalk between GPCRs and insulin/IGF receptor systems.

Insulin/IGF-1 receptor signaling and pancreatic cancer. Cellular responses to insulin have been extensively characterized in diverse tissues, including liver, muscle, and fat (23, 24), but the signaling pathways activated by insulin/IGF-1 receptors in pancreatic cancer cells are less completely understood. In most cell types, binding of insulin to its tetrameric receptor results in receptor autophosphorylation and activation of the receptor tyrosine kinase, followed by tyrosine phosphorylation of insulin receptor substrates (IRS 1-4), which further propagate downstream signals via assembly of signaling complexes, notably with the p85 regulatory subunit of PI3K (25, 26). The highly related IGF-1 receptor (IGF-1R) can also be activated by insulin (26), in particular at the high concentrations of this hormone present in the pancreatic microenvironment. Furthermore, IGF-1 produced by pancreatic stromal cells in response to factors released by the cancer cells, including hedgehog, can also interact with insulin/IGF-1 receptors in these cells (27). Accordingly, pancreatic cancer cells express insulin and IGF-1 receptors and overexpress IRS-1 and IRS-2 (28, 29), underscoring the importance of these pathways in promoting pancreatic cancer proliferation.

A key insulin-IGF/1R-induced pathway via IRS in most cell types is the PI3K/Akt/mTORC1 signaling module (26). This pathway plays a pivotal role in the proliferation and survival of pancreatic cancer cells (30) and is activated in pancreatic cancer tissues (31). Products generated by PI3K activate 3-phosphoinositide-dependent kinase (PDK1), which in turn phosphorylates and activates Akt. Interestingly, the Akt2 gene is amplified or activated in a subset of pancreatic carcinomas. In contrast, activating mutations in upstream elements of the PI3K pathway (either in *PIK3CA* or *PTEN*) have not been detected in pancreatic cancer (32). It is conceivable that stimulation of PI3K by intrapancreatic insulin/IGF-1 in combination with activated KRAS (see below) is sufficient to stimulate potently the PI3K pathway and remove evolutionary selection for mutations in *PIK3CA* or *PTEN* (33).

An important downstream target of the PI3K/AKT pathway is mTOR, which integrates and transmits signals from a diverse array of signaling pathways to regulate cell survival and growth through changes in mRNA translation, ribosomal biogenesis, autophagy, and metabolism (34, 35). mTOR functions as a catalytic subunit in two distinct multiprotein complexes, mTORC1 and mTORC2 (34). mTORC1, a complex of mTOR, the substrate binding subunit raptor, GβL, and PRAS40, is responsible for the phosphorylation and regulation of 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 as 4EBP1 (25, 36). mTORC1

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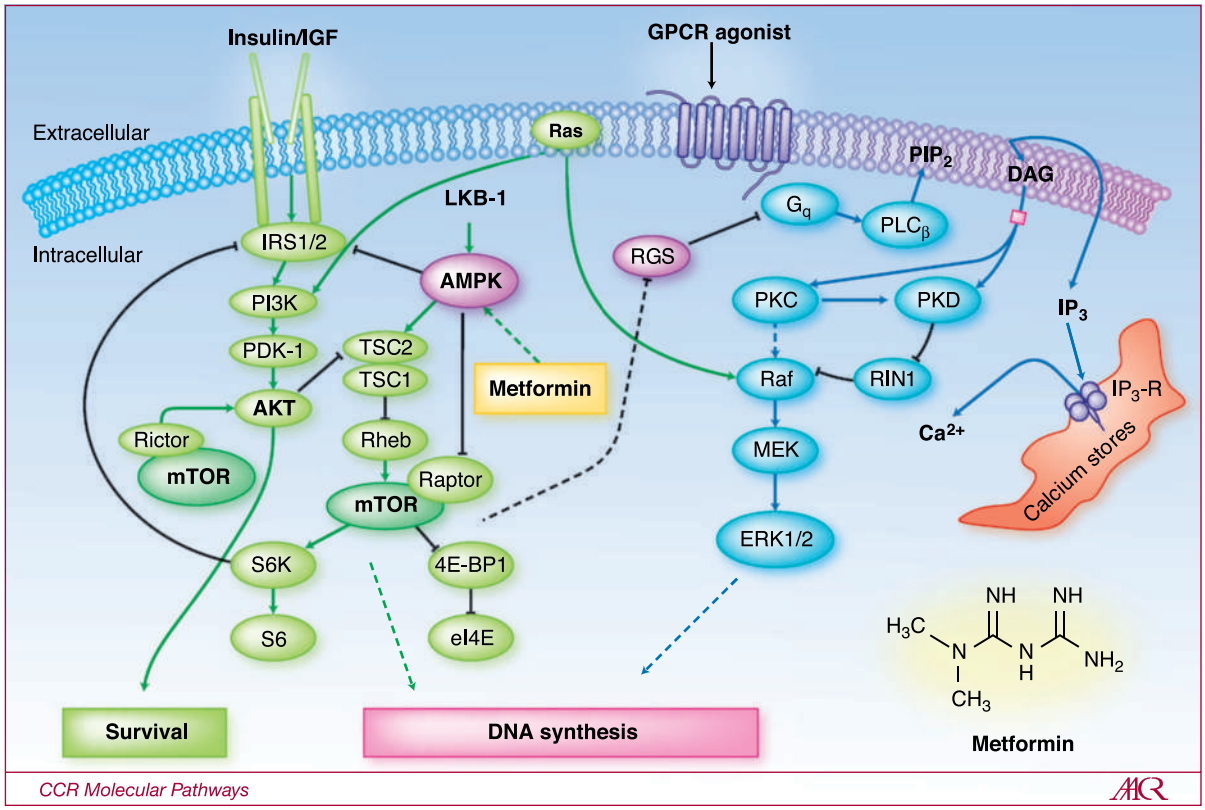

Fig. 1. Signal transduction pathways and crosstalk activated by insulin/IGF-1 receptor and GPCR systems. The binding of an agonist to its cognate GPCR induces Gq/PLC activation, hydrolysis of PIP_2, generation of Ins(1,4,5)P_3, and Ca^{2+} mobilization as described in the text. DAG, the other product of PLC, activates novel PKCs (δ, ε, θ, η,) and, in synergy with Ca^{2+}, conventional PKCs (α, β1, β2, γ). PKD (PKD1, PKD2, and PKD3) operate downstream of DAG and PKCs and lead via inactivation of the Ras/Raf inhibitor RIN1 to ERK pathway activation, potentiating signaling via mutated KRAS. Pathways activated by a typical Gq-coupled receptor are shown in blue. For the sake of clarity, stimulation of mTORC1 by ERK is not indicated in the scheme but is discussed in the text. Insulin/IGF induces PI3-kinase/Akt/TSC/Rheb/mTORC1 pathway, indicated in green. Positive crosstalk to GPCR-induced Ca^{2+} signaling is indicated by the black broken line. A plausible target are regulators of G proteins (RGS), which accelerate G protein inactivation by enhancing their GTPase activity. Negative feedback from S6K and mTORC1 on IRS is also indicated (black solid line). Metformin (chemical structure in the insert) is shown to activate AMPK (broken line) because it does not interact directly with AMPK but increases the level of 5′ AMP in the cell. AMPK opposes mTORC1 by phophorylating TSC2 and raptor, as indicated. AMPK also phosphorylates IRS (on Ser^{789}), thereby moderating its activation when the negative feedback is removed by mTORC1 inhibition. See text for further details. Another phase I trial will examine side effects and best dose of metformin when given together with temsirolimus in treating patients with metastatic or unresectable solid tumor or lymphoma.

is potently inhibited by rapamycin, whereas mTORC2, which consists of mTOR, rictor, GβL, and Sin1, is not inhibited by short-term treatment with this agent (35, 37).

There have been important advances in understanding the regulation of mTORC1 signaling. The heterodimer of TSC2 (tuberin) and TSC1 (hamartin) represses mTOR activity by acting as the GTPase-activator protein for the small G protein Ras homolog enriched in brain (Rheb), a potent activator of mTORC1 when present in a GTP-bound state (38, 39). Phosphorylation of TSC2 by Akt on Ser^{939} and Thr^{1462} (or by ERK/p90RSK on Ser^{664} and Ser^{1798}), suppresses its GTPase activity toward Rheb leading to Rheb-GTP and thereby to mTORC1 activation. In addition, Rheb-GTP stimulates phospholipase D1 (PLD1) to generate phosphatidic acid, a positive effector of mTORC1 activation (40) and an alternative route to DAG generation (1). Recent evidence obtained with pancreatic cancer cells suggests that mTORC1 has a hitherto unrecognized function in cellular regulation, namely mediation of crosstalk with other signal transduction pathways leading to heightened cellular responsiveness to GPCR agonists (2, 8).

Insulin/IGF-1 and GPCR signaling pathways crosstalk through mTORC1. Although the signaling pathways activated by *either* GPCR agonists *or* insulin/IGF-1 have been the subject of intense scrutiny (1, 23, 24, 41), much less is known about the mechanisms by which these pathways crosstalk in pancreatic cancer cells or in any other cell type. Crosstalk between different signaling pathways occurs at the most proximal level (i.e., receptor to receptor) as well as at a variety of loci further downstream in signaling cascades.

Recently, we identified a novel crosstalk between insulin/IGF-1 receptors and GPCR signaling systems in pancreatic cancer cells (2, 8). Exposure of these cells to physiological concentrations of insulin rapidly augmented early signals, including intracellular Ca^{2+} mobilization, in response to multiple agonists of Gq-coupled receptors

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(2, 8). Insulin-induced potentiation of GPCR signaling was prevented by inhibitors of PI3K or by the specific mTORC1 inhibitor rapamycin. These results indicate that the PI3K/Akt/mTORC1 pathway mediates a novel crosstalk between insulin receptor and GPCR signaling systems (2). In turn, GPCRs induce mTORC1 activation via ERK/p90RSK rather than PI3K/Akt, implying that mTORC1 is a point of convergence and amplification in the signaling network induced by insulin/IGF-1 and GPCR agonists.

Further results show that insulin/IGF-1 also crosstalks with GPCR agonists to promote long-term biological responses in pancreatic cancer cells, including DNA synthesis, proliferation, and anchorage-independent growth (8). We hypothesize that the concomitant activation of the PI3K/Akt pathway (via insulin/IGF-1 receptor), the Gq/PLC/PKD/ERK pathway (via agonist-induced Gq signaling and enhanced by insulin/IGF-1 signaling), and mTORC1 (synergistically by insulin/IGF-1-induced PI3K/Akt and GPCR-stimulated ERK/p90RSK) in pancreatic cancer cells potently stimulates DNA synthesis and proliferation of these cancer cells. In addition, Akt and ERK phosphorylate other targets implicated in pancreatic cell survival and expression of cell cycle genes. Because both the ERK and PI3K pathways are well known effectors of KRAS (42), the activating mutation of KRAS (apparent in 90% of pancreatic cancers) is likely to reinforce the crosstalk between insulin/IGF-1 receptor and GPCR signaling systems, thereby increasing the robustness of the network induced by insulin/IGF-1 and GPCR agonists in pancreatic cancer cells. Thus, crosstalk between these signaling pathways operates at multiple levels and stimulates anchorage-dependent and anchorage-independent proliferation of pancreatic cancer cells.

Clinical-Translational Advances

Metformin disrupts crosstalk between insulin/IGF-1 and GPCR signaling. The biguanide metformin (1,1-dimethylbiguanide hydrochloride, Fig. 1) is the most widely prescribed drug for treatment of type 2 diabetes in the world. Although it has been in clinical use for decades, its precise molecular mechanism of action remains incompletely understood. The primary systemic effect of metformin is the lowering of blood glucose levels through reduced hepatic gluconeogenesis and increased glucose uptake in peripheral tissues, including skeletal muscles and adipose tissue (43). Metformin not only lowers blood glucose but also reduces the hyperinsulinemia associated with insulin resistance.

At the cellular level, metformin is known to stimulate AMP-activated protein kinase (AMPK) activation (44). AMPK is a conserved regulator of the cellular response to low energy, and it is activated when ATP concentrations decrease and 5′-AMP concentrations increase in response to nutrient deprivation, hypoxia, or metformin administration (45). AMPK exists as a heterotrimer, composed of the catalytic kinase α subunit and two associated regulatory subunits, β and γ (45). AMP directly binds to the AMPK γ subunit, causing a conformational change that exposes the critical threonine (Thr^{172} in human AMPK) in the activation loop of the α subunit. The ubiquitously expressed and evolutionarily conserved serine-threonine kinase liver kinase B1/serine-threonine kinase 11 (LKB-1/STK11) represents the major (but not the only) kinase phosphorylating the AMPK activation loop under conditions of energy stress (46). LKB-1/STK11 is mutated in the Peutz-Jegher syndrome (45), characterized by predisposition to gastrointestinal neoplasms, including pancreatic adenocarcinoma.

In contrast to rapamycin, AMPK inhibits mTORC1 at multiple levels: (1) AMPK stimulates TSC2 function via phosphorylation on Ser^{1345} (47–49), leading to accumulation of Rheb-GDP (the inactive form) and thereby to inhibition of mTORC1 activation; (2) AMPK also inhibits mTORC1 signaling by direct phosphorylation of raptor (on Ser^{722} and Ser^{792}), which disrupts its association with mTOR (50).

Because our studies identified mTORC1 as a site of signaling crosstalk, we examined whether metformin-induced AMPK activation opposes interaction between insulin/IGF-1 receptor and GPCR signaling pathways in pancreatic cancer cells. We found that metformin prevented crosstalk between insulin/IGF-1 receptor and GPCR signaling systems on Ca^{2+} mobilization, mTORC1 activation, DNA synthesis, and proliferation in a variety of pancreatic cancer cell lines (see ref. 8 for details). We concluded that metformin disrupts crosstalk between insulin/IGF-1 and GPCR signaling systems through AMPK in human pancreatic cancer cells.

Further studies showed that metformin administration markedly inhibited the growth of human pancreatic cancer cells xenografted in nu/nu mice (8). In addition, metformin prevented carcinogen-induced pancreatic acinar cancer in hamsters maintained on high-fat diets (51) and inhibited the proliferation of breast and p53-/- colon cancer cells in preclinical models (52–54). A recent study suggested that breast cancer stem cells, with activated mTORC1 pathway, were selectively targeted by metformin *in vitro* and in a xenograft mouse model (55). All these studies suggest that the antidiabetic drug metformin provides a novel therapy for pancreatic cancer and other insulin-related tumors, including those from colon and breast.

Type 2 diabetes mellitus is associated with pancreatic cancer and metformin administration reduces the risk of cancer. A large number of epidemiological studies linked long standing type 2 diabetes, obesity, and metabolic syndrome with *increased* risk for developing a variety of clinically aggressive cancers, including pancreatic and colon cancer (56). These metabolic conditions are characterized by peripheral insulin resistance, compensatory overproduction of insulin by the β cells of the islet, and increased bioavailability of IGF-1. Given the complexity of the pancreatic portal microcirculation (57) and the close topographical relationship between the islets and small ducts, it is conceivable that locally overproduced insulin acts directly on insulin/IGF-1 receptors expressed by ductal cells with KRAS mutation, stimulating crosstalk with GPCRs also expressed by these cells.

Remarkably, recent epidemiological reports linked administration of metformin with a *reduced* risk of cancer in diabetic

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patients (reviewed in ref. 58). For example, an observational cohort study involving patients with type 2 diabetes mellitus who were new users of metformin showed a 37% *adjusted* reduced risk of cancer as compared with patients with diabetes, who had never used metformin (59). Other studies observed a lower cancer-related mortality rate in patients with type 2 diabetes who received metformin as compared with other treatments, including exogenous insulin or sulfonylureas (58). Strikingly, a recent study showed that diabetic patients who had taken metformin had a 62% lower adjusted incidence of pancreatic cancer compared with those who had not taken metformin (60). In contrast, diabetic patients who had taken insulin or insulin secretagogues (e.g., sulfonylureas) had a significantly higher risk of pancreatic cancer compared with diabetic patients who had not taken these drugs (60). Although epidemiological associations do not establish causation, these studies linked administration of metformin with a reduced incidence and improved prognosis in cancer patients, including pancreatic cancer.

Cinical trials. There are many interventional clinical trials (completed, active, or in recruiting phase) testing the efficacy of metformin, either alone or in combination with other drugs, in patients with type 2 diabetes mellitus and other conditions, including insulin resistance, hyperinsulinemia, obesity, polycystic ovary syndrome, atherosclerosis, hypertension, dyslipidemias, and nonalcoholic steatohepatitis. These trials see http://clinicalTrials.gov for details offer a wealth of information about the safety and tolerability of metformin in healthy subjects or in patients with type 2 diabetes mellitus and other conditions. In view of the evidence obtained with cells in culture and preclinical animal models, as well as the epidemiological studies linking metformin administration with reduced risk of cancer in diabetic patients, a number of interventional clinical trials are being developed in different countries to test the effects of metformin in human cancers. Most of them are in the recruiting phase. Three independent studies in the United States (sponsored by the MD Anderson Cancer Center, the University of Columbia, and the Vanderbilt-Ingram Cancer Center in collaboration with the National Cancer Institute), another sponsored by Mount Sinai Hospital, Canada, and a phase II study, sponsored by the Seoul National University Hospital, Korea, will be testing metformin in patients with breast cancer who are about to undergo surgery to determine cell proliferation rates and signaling biomarkers in tumor tissue. A phase II study, sponsored by the University Health Network, Toronto, is recruiting participants for investigating the effect of metformin therapy on the proliferation of prostate cancer cells prior to radical prostatectomy. A phase I trial, sponsored by the London Regional Cancer Program at London Health Sciences Centre, will examine side effects and best dose of metformin when given together with temsirolimus (a rapamycin analog) in treating patients with metastatic or unresectable solid tumors or lymphoma.

Despite its enormous clinical implications, it should be emphasized that there is no complete understanding of the precise molecular mechanism by which metformin inhibits the proliferation of cancer cells. Further research is required to clarify the extent to which relevant doses of metformin act to inhibit the respiratory chain, stimulate AMPK in cancer cells, and elucidate the contribution of AMPK-dependent pathways, including inhibition of crosstalk signaling, to its antineoplastic effects. These topics require further investigation before large-scale (phase III-IV) clinical trials can be initiated.

Conclusions

The current paradigm of pancreatic cancer development is that activated KRAS serves to initiate precursor lesions, including pancreatic intra-epithelial neoplasias. Subsequent progressive accumulation of pro-oncogenic mutations during the promotional phase of pancreatic tumorigenesis requires sustained cell proliferation. We propose that the crosstalk between insulin/IGF-1 and GPCR signaling pathways, reinforced by KRAS activation, plays a role in the promotional phase of these initiated cells and provides a novel target for therapeutic intervention. Because insulin/IGF-1 and GPCR signaling are implicated in autocrine-paracrine stimulation of other malignancies, a similar crosstalk mechanism may be operative in the promotional phase of other cancer cell types.

Because metformin-induced activation of AMPK inhibits mTORC1 function at multiple levels, it is plausible that metformin inhibits pancreatic cancer growth via AMPK-mediated inhibition of mTORC1 activation (Fig. 1). Given that insulin/IGF-1 seems to play a critical role in pancreatic cancer development and that GPCRs are implicated as autocrine-paracrine signals in this process, it is of interest that metformin disrupted crosstalk between insulin/IGF-1 and GPCR signaling pathways. We posit that sites of positive crosstalk between signaling pathways activated in cancer cells are critical targets for cancer therapy.

U.S. Food and Drug Administration (FDA)-approved drugs have usually favorable pharmacological properties and safety profiles. Unexpected biological activities of such well-characterized inhibitors, therefore, represent particularly exciting possibilities for rapid translational research. Given the growth-inhibitory effects of metformin on pancreatic cancer cells *in vitro* and *in vivo*, we hypothesize that this agent, widely used in type 2 diabetes, can offer a novel approach for the prevention and treatment of pancreatic cancer and other solid malignancies.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Grant Support

Work in the laboratory of E. Rozengurt is supported by NIH grants R21CA137292, R01 DK56930, R01 DK55003, and P30 DK41301.

Received 02/10/2010; revised 02/17/2010; accepted 02/18/2010; published OnlineFirst 04/13/2010.

Published OnlineFirst April 13, 2010; DOI: 10.1158/1078-0432.CCR-09-2229

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Published OnlineFirst April 13, 2010; DOI: 10.1158/1078-0432.CCR-09-2229

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Clin Cancer Res 2010;16:2505-2511. Published OnlineFirst April 13, 2010.