

Perspective

Perspective on Bao et al., p. 355

Metformin and Cancer Stem Cells: Old Drug, New Targets

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Abstract

In this issue of the journal, Bao and colleagues report (beginning on page 355) that the antidiabetic drug metformin targets pancreatic cancer stem cells through, at least partially, the modulation of miRNA expression and subsequent regulation of stem cell renewal and signaling factors. In this Perspective, we briefly discuss the cancer stem cell hypothesis, its clinical relevance, and how targeting the mTOR pathway may yield an avenue for disrupting the cancer stem cell compartment and thus yield long-term therapeutic benefit in multiple cancers. *Cancer Prev Res*; 5(3); 351–4. ©2012 AACR.

The cancer stem cell (CSC) hypothesis of tumorigenesis has developed out of our understanding of the functional heterogeneity observed in human tumor cells. On the basis of this hypothesis, CSCs sit at the top of a tumor "developmental" hierarchy and have 2 key characteristics—self-renewal and the ability to give rise to the full spectrum of phenotypic progeny of a particular tumor. We have known for decades that not all tumor cells are created equal, but rigorous experimental characterization of this hierarchy and the concept of CSCs did not occur until 1994, when John Dick and his group defined the CD34⁺/CD38[−] subpopulation of primary human acute myeloid leukemia cells as the tumor-initiating population (1). Subsequent work carried out by multiple groups experimentally verified the CSC hypothesis, first in breast tumors and subsequently in other solid malignancies (2). Pancreatic CSCs were initially defined in 2007 by Li and colleagues using the markers CD24, CD44, and epithelial-specific antigen (ESA/epithelial cell adhesion molecule (EpCAM; ref. 3). Subsequent work by Hermann and colleagues (4) used the stem cell marker CD133 (prominin-1) and CXC chemokine receptor type 4 (CXCR4) to delineate 2 functionally distinct pancreatic CSC subsets. Additional pancreatic CSC marker profiles have since been defined and include aldehyde dehydrogenase 1 (ALDH1) and c-Met expression (5, 6).

CSCs are thought to be very important in the clinical treatment of cancer. Current therapeutic approaches for solid malignancies rely on a combination of surgical resection, targeted radiotherapy, and systemic chemotherapy. Surgical approaches are usually successful only when the cancer is detected at an early stage and remains localized

without any occult or gross metastatic spread. Only a minority of patients (~10%–15%) fall into this category in the setting of pancreatic cancer. As a result, systemic chemoradiotherapy is the backbone of pancreatic cancer treatment for most patients. Unfortunately, although experimental evidence from preclinical models and treated clinical samples has shown that standard protocol chemotherapeutic regimens lead to bulk tumor cell death, these regimens also enrich the remaining live tumor population for resistant CSCs. Bao and colleagues showed that glioma CD133⁺ CSCs preferentially survive targeted radiotherapy through the upregulation of the DNA damage–response pathway (7). Colon CSCs show resistance to systemic therapies partially through interleukin-4–dependent mechanisms (8, 9). A breast cancer cohort study showed upregulation of the CSC population after systemic chemotherapy by comparing pretreatment and posttreatment tumor biopsies (10). The concept of CSC enrichment following chemoradiotherapy has clear clinical implications for the patient. Chemotherapy regimens have a defined treatment period, and so once they are stopped, the tumor often recurs as the core tumor-initiating cell population remains untreated. Recent work showed that stem cell antigen-1 (Sca-1) negatively regulates PPAR γ function in breast cancer cells. Therefore, Sca-1–expressing cells (i.e., stem cells) should be less sensitive to the antineoplastic effects of PPAR γ agonists. Unfortunately, this means that chemoprevention with these drugs again potentially misses the CSC compartment. Also, thiazolidinediones, which are a class of PPAR γ ligands, may have an increased risk of pancreatic ductal adenocarcinoma development. These data support the ongoing efforts to find therapeutic approaches that would specifically target the CSC compartment of a tumor.

The mTOR pathway is a key pathway that is dysregulated during tumor growth and can serve as a therapeutic and preventive target to attack neoplastic cells. In healthy cells, mTOR signaling integrates multiple inputs from the cell environment via growth factor receptors together with the internal metabolic state of the cell (reviewed in ref. 11). Growth factors use receptor-associated tyrosine kinases to

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activate a host of downstream signaling components, including the Ras/Raf/mitogen-activated protein kinase pathway and the phosphoinositide 3-kinase (PI3K)/Akt pathways. Both of these pathways directly inhibit the function of the tuberous sclerosis complex (TSC; comprising TSC-1 and -2) by phosphorylating TSC-2, which in turn leads to the activation of the mTOR complex 1 (mTORC1) by the GTPase Rheb (11). Once active, mTORC1 phosphorylates multiple downstream targets including 4E-binding protein 1 (4EBP1) and S6 kinase, further activating translation of growth factors and cell-cycle regulators and amplifying ribosome biogenesis. In addition to the TSC, mTOR signaling is negatively regulated by the liver kinase B1 [LKB1 or serine/threonine kinase 11 (STK11)]/AMP kinase (AMPK) pathway. LKB1 was originally defined as the kinase regulating AMPK to mediate glucose homeostasis (12). AMPK also responds to the relative levels of ATP/AMP inside the cell, which serve as a measure of the cellular energy state. Elevated levels of AMP lead to the activation of the AMPK pathway, which in turn leads to direct phosphorylation of the TSC and the upregulation of its activity, resulting in mTOR inhibition (11). The importance of the mTOR signaling pathway in cancer is underlined by the fact that mutations in multiple regulators are associated with familial neoplastic syndromes such as tuberous sclerosis (mutations in TSC-1 and TSC-2) and Peutz-Jeghers syndrome (mutations in LKB1). Indeed, the potential efficacy of mTOR inhibitors in patients with Peutz-Jeghers syndrome who develop pancreatic cancer was reported recently (13).

mTOR pathway dysregulation has also been implicated in stem cell biology. Zhou and colleagues used breast cancer cell lines to test the involvement of the mTOR pathway in breast cancer stem cell survival (14). Using the side-population (SP) cell selection criterion for CSCs, they showed that rapamycin, a direct mTORC1 inhibitor, and short hairpin RNA (shRNA) inhibition of mTOR expression decreased the fraction of CSCs in the MCF-7 cell line, thereby inhibiting colony formation and *in vivo* tumorigenicity. Phosphatase and tensin homolog (*Pten*) deletion leads to the eventual exhaustion of normal hematopoietic stem cells (HSC) because of their unregulated proliferation (15). Contrary to this finding, *Pten* deletion in leukemia-initiating cells allows the cells to continue to proliferate without any difficulty, resulting in tumor growth in engrafted murine hosts. This loss of self-renewal regulation in the leukemia-initiating cells was tied to the aberrant activation of mTOR, and rapamycin depleted the leukemia-initiating cells (15).

Inhibition of mTOR by rapamycin has also been used in a preclinical model of pancreatic cancer (16). Gemcitabine treatment of bulk pancreatic cancer cell lines or cells derived from primary human pancreatic tumors led to the enrichment of the CD133⁺ CSC population. Combining gemcitabine with rapamycin and cyclopamine, a hedgehog pathway inhibitor, highly suppressed CSC survival. This suppression correlated with decreased rates of tumor implantation and metastasis and significantly prolonged the survival of mice with orthotopic xenografts. A

particularly significant result was a lack of tumor recurrence even after the cessation of systemic therapy (but within the experimental time period—mice were not followed out to natural death), suggesting that a combination therapy including an mTOR inhibitor may prove to be highly effective against the CSC population in pancreatic cancer.

Metformin belongs to the biguanide class of antidiabetic drugs and is of emerging interest for cancer prevention and therapy (17). It serves as first-line therapy in type 2 diabetes mellitus, which is associated with tissue resistance to the action of insulin. It has been used widely for several decades, but its mechanism of action was unclear until recently. In 2005, Shaw and colleagues showed that metformin exerts its action through the activation of the LKB1/AMPK axis and thereby indirectly inhibits the mTORC1 complex (12). Additional clinical follow-up has now showed that metformin may have profound effects on tumor initiation and progression (18). Initial evidence for anticancer effects came from a case-control study in Scotland showing that metformin reduced the overall incidence of cancer by 15% to 20% in diabetic patients (19). The effect was time- and dose-dependent, with steadily declining cancer development in patients taking higher metformin doses for longer periods. A subsequent case-control study from MD Anderson Cancer Center, Houston, TX, showed that specific antidiabetic medications had distinct effects on the risk of pancreatic cancer (20). Metformin had an adjusted OR of 0.38 [95% confidence interval (CI), 0.21–0.67] for the occurrence of pancreatic cancer, whereas the risk of pancreatic cancer stayed the same or increased with thiazolidinediones and insulin. A meta-analysis published in a recent issue of this journal also showed a correlation between metformin use and pancreatic cancer risk reduction, as well as showing similar trends for other sites of malignancy including the colon, breast, liver, and prostate (21).

Evidence supporting the antineoplastic activity of metformin has also been seen in preclinical models of cancer. Schneider and colleagues showed that metformin partially prevented the formation of premalignant pancreatic lesions and completely abrogated the development of full-blown carcinomas in a hamster model of chemically induced pancreatic cancer (22). Metformin also reduced the growth of a breast cancer cell line in an AMPK-dependent manner, with downstream inhibition of mTOR signaling and protein translation (23, 24). Metformin inhibition of CSCs was first shown in 2009 in preclinical breast cancer models (25). This study showed that metformin inhibited colony and mammosphere formation in breast cancer cell lines in a dose-dependent manner. Doxorubicin, a standard component of breast cancer chemotherapy, produced a negligible effect on the proportion of CD44⁺/CD24^{low} CSCs in the remaining live cells, whereas metformin alone or in combination with doxorubicin significantly reduced the number of surviving CSCs. More important, doxorubicin plus metformin produced a durable regression of tumors in nude mice with tumor xenografts, even after cessation of

therapy, similar to rapamycin results in the preclinical model of pancreatic cancer discussed earlier (16). These results were subsequently extended to cancer cell lines from prostate and lung adenocarcinomas, where metformin similarly inhibited CSCs (26). Taken together, the results from these preclinical systems and clinical studies highlight the potential efficacy of metformin and other mTOR inhibitors in the prevention and treatment of multiple malignancies. Despite ongoing research, however, we still have a very limited understanding of the molecular mechanisms underlying metformin and other mTOR inhibitor effects in tumor suppression and CSC targeting.

Work reported by Bao and colleagues (27) in this issue of the journal expands our understanding of the use of metformin in targeting pancreatic CSCs and begins to delineate some of the mechanistic details underlying metformin suppression of CSCs. The authors derived chemotherapy-resistant isogenic versions of the pancreatic cell lines AsPC-1 and MiaPaCa-2 through prolonged, intermittent exposure to gemcitabine and erlotinib (Tarceva), an epidermal growth factor receptor inhibitor. Metformin inhibited *in vitro* colony formation and invasion in a dose-dependent manner in all 4 tested cell lines. The authors also found that the drug, alone or in combination with a curcumin derivative, inhibited tumorsphere formation, a surrogate for stem cell self-renewal capacity. These results suggest that metformin at least partially abrogated the function of the CSC subpopulation in these cell lines. Although no *in vivo* tumor growth data were presented, the work has clear parallels to that in the breast, prostate, and lung cancer systems mentioned earlier. It will be important to verify the biologic relevance of the *in vitro* findings of the current study by extending them to *in vivo* preclinical models of pancreatic cancer.

The novel findings of this work concern the molecular mechanisms underlying the ability of metformin to target the CSC compartment. The authors note that metformin treatment led to a decrease in the mRNA levels of Nanog and Oct4, 2 transcription factors that were originally defined as a part of the self-renewal/maintenance machinery for embryonic stem cells (28, 29). They also noted decreased expression of Notch1 and enhancer of zeste homolog 2 (EZH2) mRNAs. Notch signaling has been previously implicated in stem cell signaling (30), and EZH2 is the methyltransferase component of the Polycomb repressor complex 2, which mediates the silencing of genes involved in cell differentiation (31). At least part of this gene regulation by metformin occurred through miRNA-mediated control of mRNA tran-

scripts, notably involving let-7 and the miR-200 family of miRNAs. The let-7 family has been previously linked to the regulation of Ras signaling (32), and the miR-200 family directly controls factors involved in epithelial–mesenchymal transition and maintenance of the stem cell state (33). Together, these results point to the ability of metformin to directly modulate the levels of key regulators of stem cell function by altering levels of multiple regulatory switch miRNAs.

Several cancer model studies have shown functional inhibition of the CSC compartment by the disruption of mTOR signaling (14–16) and thus suggest a direct link between mTOR signaling and maintenance of the CSC compartment. Studies such as the current work by Bao and colleagues (27) will help to define the molecular mechanisms linking the mTOR and other signaling pathways with the functional CSC state in a tumor. In targeting the CSC compartment responsible for tumor growth and recurrence, this important work has the potential to define new cancer therapy and prevention targets.

The key remaining question is how best to rapidly translate the data on mTOR inhibitor effects on CSCs into new approaches for preventing and treating pancreatic cancer and other malignancies. Mounting evidence from the present *in vitro* work of Bao and colleagues (27) and other published preclinical studies suggests that metformin may not only play a role in cancer prevention but also may serve as an excellent addition to combinations for cancer therapy. One advantage of using metformin is that several decades of use and study have defined the side effect profile of metformin very well, making it attractive for moving forward into phase II/III clinical trials. Indeed, multiple clinical trials of mTOR inhibitors in multiple malignancies have recently been completed or are actively accruing patients. Along with other studies showing the efficacy of metformin in targeting CSC populations in multiple tumor types, the current work supports further testing of metformin in the clinical setting.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

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