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Role of Dietary Cancer-Preventive Phytochemicals in Pancreatic Cancer Stem Cells

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Abstract

Purpose of review: This article provides a brief overview of cancer-preventive phytochemicals specifically targeting pancreatic cancer (PC) stem cells for prevention and treatment.

Recent findings: Cancer stem cells (CSCs) represent a small proportion of the total cells of a given tumor, and contribute to tumor growth, recurrence, metastasis, and treatment resistance. Many intertwined pathways, including hedgehog, Wnt Signaling, and NOTCH, have been shown to play a role in the formation of CSCs. Recently, numerous chemopreventive agents, such as genistein, (–)-epigallocatechin-3-gallate (EGCG), sulforaphane, curcumin, resveratrol, and quercetin, have been shown to target CSCs mediated through the inhibition of multiple signalling pathways, to avoid toxicity and the side effects of chemical compounds.

Summary: A growing body of research suggests that CSCs are the drivers in treatment resistance, cancer recurrence, and metastasis, in addition to tumor initiation and heterogeneity. Patient survival depends on these CSCs, which are one cause of tumor recurrence after surgery and chemotherapy. Therefore, target selection; an improved understanding of CSC biology, the genetic and molecular profiles of CSCs, and their key signaling pathways, and; appropriate clinical trials endpoints that are designed to target CSCs will help in the development of drugs that will specifically target this small population of stem cells.

Keywords

pancreatic cancer; phytochemicals; cancer stem cells; polyphenols

Introduction

PC is the third leading cause of cancer death in the United States [1]. This malignancy accounts for about 3% of all cancers in the US and about 7% of all cancer deaths. The most common type of pancreatic cancer is exocrine cancer, of which about 95% is pancreatic ductal adenocarcinoma (PDAC) [2]. Despite recent advances in the molecular and biological understanding of the disease, with different treatment strategies developed in the few decades, the five-year survival rate of PC is 8.2% [1], suggesting that patients diagnosed with PC have high mortality and extremely poor prognosis.

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Incidence and Prevalence

PC is more common with increasing age, and is diagnosed among people aged 65–74. The median age at diagnosis is 70. Based on 2012–2014 data, approximately 1.6% of men and women will be diagnosed with pancreatic cancer at some point during their lifetime. The number of new cases of pancreatic cancer was 12.5 per 100,000 men and women per year from 2010–2014. In 2018, about 55,440 people will be diagnosed with and about 44,330 people will die from pancreatic cancer [3]. Globally, incidence and mortality rates of pancreatic cancer are 4.9 and 4.8 per 100,000, respectively [4]. Due to poor survival, the numbers of individuals who are diagnosed and who die of pancreatic cancer are almost the same each year.

Risk factors, Clinical Symptoms, Diagnosis, and Therapy

Risk Factors:

PC is a silent disease and is rarely diagnosed early. When it is detected, the cancer is usually surgically unresectable, which is one of the reasons for the poor survival rate and high mortality. Behavioral and environmental risk factors have been associated with pancreatic carcinogenesis. Cigarette smoking is the best known and a well-established environmental risk factor for PC development [5, 6]. In fact, cigarette smoking may be responsible for approximately 20% of PC cases [7]. Other well-known risk factors, such as family history of PC, obesity, alcohol consumption, chronic pancreatitis, diabetes, race (African American population is more prone), diet (rich in meats, cholesterol, fried foods, nitrosamines, and other chemicals), and other forms of tobacco use are associated with the development of PC [8–12].

Clinical Symptoms and Diagnosis:

PC is difficult to detect and diagnose for the following reasons: 1) absence of noticeable signs or symptoms in the early stages of PC; 2) signs of PC, when present, are similar to the signs of many other illnesses, such as pancreatitis or an ulcer; and 3) the pancreas is obscured by other organs in the abdomen and is difficult to visualize clearly on imaging tests. When symptoms appear, the type of symptom depends on the site of the tumor within the pancreas and the degree of tumor involvement. By the time symptoms develop, PC has often spread outside the pancreas. Early-stage PC typically causes vague, nonspecific symptoms, including poor appetite, weight loss, abdominal or back pain, blood clots, and, occasionally, the development of diabetes. Tumors that develop near the bile duct can cause jaundice (yellowing of the skin and eyes), which sometimes facilitates an early diagnosis [13].

Treatment:

Surgery, radiation, chemotherapy, targeted therapies (against growth factors like EGFR, angiogenesis factors, drugs that target the tumor stroma and cancer stem cells), and immune therapy are common treatment methods that may extend survival to some extent. Less than 20% of patients are candidates for surgery because PC is usually detected after it has spread beyond the pancreas. For those who undergo surgery, adjuvant treatment with chemotherapy,

and sometimes radiation, is prescribed. For those with advanced disease, chemotherapy, sometimes along with a targeted therapy drug, may lengthen survival. Clinical trials are testing several new agents for their ability to improve survival. A combination chemotherapy regimen of oxaliplatin, irinotecan, FU, and leucovorin (FOLFIRINOX) is now recommended as the first-line treatment for patients with metastatic pancreatic cancer [14, 15].

It is common to see poor drug responses or resistance after repeated treatments of non-targeted chemotherapy in patients with advanced malignancies, but the underlying causes are still not well understood. Although several aspects of PDAC have been studied, the evidence for the emergence of PC from cancer stem cells has been limited, although intriguing. One rationale for studying cancer-initiating or cancer stem cells is to determine whether there is a rare subset of cells responsible for this drug resistance problem, and to unravel the molecular mechanisms that govern their existence to better address the therapies that can be targeted towards cancer stem cells.

PC Stem Cells: Characteristics and Resistance

Both normal tissues and tumors encompass a heterogeneous collection of cell types, including immune cells, a stroma consisting of various mesenchymal and endothelial cells, and a variety of normal or malignant cells specific to the tissue. Cells within tumors often seem to correspond to different stages of development due to microenvironment influence and genomic instability. A normal cell becomes a tumor when it has undergone a transformation as a result of mutation and failure of the repair mechanism. This cell, on further mutation, becomes a transformed cell and give rise to a tumor. Therefore, the tumor-initiating cell is a normal cell that receives the first cancer-causing mutations. However, cancer stem cells (CSC) are a distinct population that can be prospectively isolated from the remainder of the tumor cells. These CSCs maintain tumor propagation and have two main properties for the establishment and recurrence of cancerous tumors: their capacity for self-renewal (one of the hallmarks of cancer and the defining characteristic of CSCs) and their potential to differentiate into unlimited heterogeneous populations of cancer cells (multipotency). Characteristic of continuous division and self-renewal is the ability to avoid fatal telomere shortening through the action of the telomerase complex [16, 17]. Functionally, these cells undergo an unlimited number of asymmetrical cell divisions, and produce at least one daughter progenitor cell with each division, which maintains their indefinite self-renewing capacity [18].

CSCs, which typically represent a small proportion of total cells of a given tumor, contribute to tumor growth, recurrence, metastasis, and treatment resistance [19–21]. However, debate regarding the origin of CSCs is ongoing. One theory suggests that spontaneous transformation of non-tumorigenic stem cells into CSCs by genomic instability is a potential source of CSCs. However, another theory posits that tissue resident stem cells acquiring a malignant phenotype by accumulation of mutations are the potential originators of CSCs.

Stem cells in the pancreas were first demonstrated by Li et al. (2007) by isolating a small population (0.2–0.8%) of cancer cells with high expression of cell surface markers (CD44, CD24, and epithelial-specific antigen [ESA]) from tumors [22]. They further showed that

these cells have 100-fold more tumorigenic potential in xenografts than do nontumorigenic cancer cells, when implanted in immunocompromised mice (100 triple-positive cells were sufficient to form new tumors in 6 of 12 experimental NOD/SCID mice). This discovery led the researchers to link pancreatic CSCs to the progressive nature of PC, its highly drug-resistant phenotype, early metastasis, and inclination to recurrence (Table 1). This landmark realization has opened the door to find the mechanisms by which pancreatic CSCs control the survival, proliferation, and invasion of PC, and to develop novel therapeutics that may specifically target the CSCs in PC.

Pathways associated with CSCs in PC

There is mounting recent evidence demonstrating the molecular understanding of CSCs. Many intertwined pathways, including hedgehog, Wnt Signaling, and NOTCH, have been shown to play a role in the formation of CSCs [23] (Figure 1). Therefore, it is imperative to address the multifaceted role of these signalling pathways in the function of CSCs to better understand the anti-cancer therapeutic agents.

The hedgehog pathway is activated by Desert hedgehog (DHH), Sonic hedgehog (SHH), or Indian hedgehog (IHH) serving as ligands for transmembrane protein. Subsequently, the Glioma Associated Oncogene homologs (GLIs) transcriptional factors are upregulated and translocated into the nucleus, thereby modulating the downstream gene expressions [24, 25]. Aberrations in the hedgehog pathway proteins have been associated with multiple cancers, including basal cell carcinoma, small cell lung carcinoma, melanoma, breast cancer, prostate cancer, and pancreatic carcinoma [24, 26–28].

The Notch pathway consists of four NOTCH transmembrane receptors 1–4, which are activated when a Notch ligand (Jagged-JAG 1,2 and Delta-like-DL 1–3) binds to the receptor and undergoes multiple cleavages by different enzymes, thereby mediating its effects by regulating target genes [29, 30]. The NOTCH pathway plays an important role in the cell differentiation process of the developing pancreas and has been implicated in the development of various tumors, including breast cancer, cervical cancer, renal cancer, prostate cancer, lung cancer, colorectal carcinoma, medulloblastoma, melanoma, and pancreatic cancer [30–33].

The Wnt signaling pathway has been reportedly involved in early developmental processes, cell differentiation, and tissue regeneration. Wnt ligand interacts with transmembrane receptors, resulting in β -catenin accumulating in the cytoplasm, which then translocates into the nucleus to activate the target genes. There is increasing evidence suggesting Wnt pathway involvement in various cancers, including colorectal cancer, breast cancer, hematopoietic stem cells, melanoma, and pancreatic cancer [34–37].

Role of polyphenols in chemoprevention of CSCs (Table 2)

Soy isoflavone (genistein)

Researchers have found that isoflavones may inhibit cancer stem cells in various cancers, including lung, ovarian, prostate, and pancreatic cancer [38–41]. It has been reported that

genistein, a natural NF- κ B inhibitor, enhances the efficacy of chemotherapeutic agents in pancreatic BxPC-3 cells. In 2011, Bao et al. reported that overexpression of FoxM1 and Notch-1 in AsPC-1 pancreatic cells led to the recovery of the EMT phenotype by activation of mesenchymal cell markers with increased sphere-forming (pancreatospheres) capacity and expression of CSC surface markers (CD44 and EpCAM), which in turn are regulated by miR-200b. Further, they observed that genistein (30 and 60 μ M) inhibited the formation of pancreatospheres with reduced expression of CSCs markers (CD44 and EpCAM). Moreover, genistein inhibited growth, clonogenicity, migration, invasion, and epithelial-to-mesenchymal transition (EMT) in PC cells, which was associated with reduced expression of CD44 and EpCAM [42]. Genistein was also found to inhibit miR-27a and, subsequently, PC cell growth, inducing apoptosis [43]. Thus, genistein modulates its effects through the cell cycle, angiogenesis, and apoptosis, thereby decreasing the growth of cancer cells. Together, these findings suggest that isoflavones could become significant agents for the prevention of tumor recurrence and/or treatment of PC with improved treatment outcomes by targeting multiple oncogenic pathways, including EMT and CSC phenotypes.

Tea polyphenols (EGCG)

Polyphenols are the most significant group of components in tea, especially the catechin group of the flavanols. The major green tea polyphenols are (–)-epigallocatechin-3-gallate (EGCG), (–)-epicatechin-3-gallate (ECG), (–)-epigallocatechin (EGC), (–)-epicatechin (EC), (+)-gallocatechin (GC), and (+)-catechin, which together may constitute 30% of the dry leaf weight. Among these, EGCG is the major polyphenol and accounts for 60% of total catechins; it is believed to be the most protective agent in green tea [44, 45]. EGCG has been shown to possess outstanding cancer chemopreventive and therapeutic potential against various cancer sites in *in vitro* cancer cells, *in vivo* tumor systems (chemically induced and genetic models), and in some human epidemiologic studies. In addition, EGCG has been shown to modulate multiple signal transduction pathways by controlling the unwanted proliferation of cells, thereby imparting strong cancer chemopreventive and therapeutic effects [46].

EGCG can effectively inhibit pluripotency maintaining transcription factors (Nanog, c-Myc, and Oct-4), thereby decreasing the self-renewal capacity of PC stem cells [47]. However, the effects were further enhanced with inhibition of Nanog. EGCG also inhibits epithelial-mesenchymal transition by decreasing Snail, Slug, and ZEB-1, diminishing the migration and invasion of PC stem cells. Moreover, EGCG inhibits Sonic hedgehog pathway proteins Bcl-2 and XIAP, and activates caspase-3, thereby inducing apoptosis [47]. Combining green tea catechin (GTC) with quercetin or sulforaphane effectively eliminates CSCs by inducing miR-let7-a and inhibiting K-ras in MIA-PaCa2 and human hTERT-HPNE immortalized pancreatic duct cells CRL-1097. In addition, a mixture of green tea catechins (GTCs) significantly inhibited viability, migration, expression of MMP-2 and –9, ALDH1 activity, and colony and spheroid formation, and induced apoptosis [48]. Recently, Kumazoe et al. reported that a combination of EGCG and a phosphodiesterase 3 (PDE3) inhibitor as a strong cGMP inducer significantly suppressed the Forkhead box O3 and CD44 axis, which are indispensable for the CSC properties of PDAC [49].

Sulforaphane

Kallifatidis et al. demonstrated that sulforaphane (SFN) inhibits pancreatic CSCs by interfering with NF- κ B anti-apoptotic signaling. In a xenograft model, they showed that SFN blocked tumor growth and angiogenesis. This effect was further enhanced in combination with tumour necrosis factor-related apoptosis-inducing ligand (TRAIL) [50]. Research on SFN along with established anti-cancer drugs has also been conducted in pancreatic and other cancers. Co-treatment of cells with sulforaphane and Sorafenib eliminated pancreatic CSCs through downregulation of NF- κ B activity [51]. In another study, researchers observed that the combination of sulforaphane with other anticancer drugs, including cisplatin, gemcitabine, doxorubicin, and 5-fluorouracil, enhanced the drugs' effects on pancreatic cancer stem cells, modulated through inhibition of aldehyde dehydrogenase-1, NOTCH-1, and c-Rel [52]. Sulforaphane (SFN) inhibited the self-renewal capacity of pancreatic CSCs, which was further enhanced by inhibition of Nanog by lentiviral-mediated shRNA expression or quercetin when used in combination in pancreatic cell lines (PANC-1, MIA PaCa-2, AsPC-1, and BxPC-3 cells). Further, SFN has been shown to induce apoptosis (by inhibiting the expression of Bcl-2 and XIAP), activating caspase-3, and downregulates epithelial-mesenchymal transition proteins β -catenin, vimentin, twist-1, and ZEB1. These data suggest that SFN, either alone or in combination with quercetin, can eliminate cancer stem cell characteristics [53].

Rodova et al., reported inhibition of sonic hedgehog-Gli interaction and the downstream proteins of the pathway by sulforaphane in pancreatic cell lines, suggesting its effectiveness in regulating the self-renewal of PC stem cells. They also observed inhibition of Bcl-2 and activation of caspase, thereby inducing apoptosis [54]. The same group published a similar finding in a xenograft tumor model, in which they observed a significant reduction in the tumor growth of orthotopically implanted primary pancreatic CSCs isolated from human pancreatic tumors into the pancreases of NOD/SCID/IL2Rgamma mice. In addition, SFN inhibited the expression of pluripotency-maintaining transcription factors Nanog and Oct-4 and angiogenic markers VEGF and PDGFR α , which are downstream targets of Gli transcription [55]. Inhibition of the Shh pathway by SFN results in a marked reduction in EMT, metastasis, and antigenic markers, with significant inhibition of tumor growth in mice [55]. The combination of quercetin, sulforaphane, and GTC effectively eliminated CSCs *in vitro* by inducing miR-let7-a and inhibiting K-ras in PC [48]. Thus, sulforaphane potentially represents an inexpensive, safe, and effective alternative for the management of PC.

Curcumin

Curcumin has been shown to exhibit anti-inflammatory, antioxidant, anticlastogenic, antiviral, and antifungal properties. In addition, the protective nature of curcumin (hepatoprotective, nephro-protective, myocardial infarction protective) is well documented. Curcumin has been reported to mediate anticancer effects by modulating multiple cell signaling pathways, such as the PI3K/AKT/Protein Kinase C (PKC) pathway, intrinsic and extrinsic pathways (for apoptosis), cell proliferation pathways (cyclin D1, c-myc), cell survival pathways (Bcl-2, Bcl-xL, cFLIP, XIAP, c-IAP1), caspase activation pathways (caspase-8, 3, 9), tumor suppressor pathways (p53, p21), death receptor pathways (DR4, DR5), mitochondrial pathways, and protein kinase pathways (JNK, Akt, and AMPK) [56–

59]. Further, curcumin modulates diverse transcription factors, inflammatory cytokines, enzymes, kinases, growth factors, receptors, and various other proteins with an affinity ranging from the pM to the mM range to regulate the abovementioned pathways *in vitro*, *in vivo*, and in human samples [56–58, 60].

It was demonstrated that curcumin magnetic nanoparticles inhibit PC cell proliferation *in vitro* (using HPAF-11 and Panc-1 cell lines) and in xenograft mouse models by inhibiting the activity of NF- κ B [61, 62]. Bao et al. demonstrated that a curcumin analog decreases pancreatic cancer stem cell function and tumor growth by modulating the histone methyltransferase Enhancer of Zeste Homolog-2 (EZH2), inhibiting vascular endothelial growth factor (VEGF) and IL-6, and downregulating miR, suggesting that curcumin exerts its effects by inhibiting cancer stem cell pathways [63–65]. Further, Shahar et al. reported that curcumin increases the effects of gemcitabine on PC cells by downregulating the COX-2 and p-ERK levels [66]. Similarly, Yoshida et al. showed that curcumin sensitized pancreatic cancer cells to gemcitabine by decreasing the EZH2 subunit of Polycomb Repressive Complex 2 (PRC2) [67]. Although recent evidence suggests advances have been made in the evaluation of the phytochemical function, there is an urgent need to understand its role in PC stem cells.

Resveratrol

Resveratrol (10–30 μ M) inhibits the self-renewal capacity of pancreatic CSCs (measured by formation of primary and secondary spheroids in suspension, and cell viability in spheroids) derived from both human primary tumors and KrasG12D mice. Inhibition of CSCs slows the growth (size and weight) and development (PanIN lesions) of PC in KrasG12D mice. Resveratrol induces apoptosis of pancreatic CSCs by activating caspase-3/7 and inhibiting the expression of Bcl-2 and XIAP. Furthermore, resveratrol decreases the pluripotency-maintaining factors (Nanog, Sox-2, c-Myc, and Oct-4) and CSCs' migration and invasion, which is partly due to Zeb-1, Slug, and Snail, all of which are markers of epithelial-mesenchymal transition. Thus, resveratrol inhibits PC stem cell characteristics in humans and Kras^{G12D} transgenic mice by inhibiting pluripotency-maintaining factors and epithelial-mesenchymal transition [68].

Quercetin

Zhou et al. demonstrated a decrease in the self-renewal property of PC stem cells using the dietary polyphenol quercetin, both *in vitro* and *in vivo* [69]. They observed that quercetin decreases aldehyde dehydrogenase-1 activity in PC and inhibits epithelial-mesenchymal transition. The observed effects were more pronounced in the presence of sulforaphane, suggesting synergized effects on the PC stem cells [69]. Different researchers have shown the same synergistic effect of sulforaphane and quercetin [70]. Appari et al. reported the complementary effects of quercetin, sulforaphane, and EGCG [71], compared with individual dietary agents, in inhibiting the self-renewal capacity of PC stem cells. Moreover, Tang et al. described enhanced effects of EGCG with quercetin on inhibiting the self-renewal capacity of PC stem cells by modulating downstream Sonic hedgehog pathway proteins [47]. Quercetin inhibited the β -catenin in PC stem cells, thereby impeding their self-renewal capacity [72].

Numerous groups have examined the role of miR in regulating the CSC population and its properties. The Herr group demonstrated induction of miR-let7-a and inhibition of K-ras with combination of quercetin, sulforaphane, and catechins [71]. Nwaeburu et al. recently reported that up-regulation of microRNA let-7c by quercetin inhibits PC progression by activating Numbl [73]. Further, quercetin-induced miR-200b-3p has been shown to regulate the mode of self-renewing divisions in PC. These findings suggest cross talk between phytochemicals, microRNAs, and different signaling pathways in the regulation of self-renewing CSC divisions [74].

Conclusions

The identification of CSCs in different cancers has opened a new door for cancer treatment. Evidence shows that CSCs, a subset of cancer cells, may hold a key to controlling cancer and potentially achieving durable clinical responses. A growing body of research suggests that CSCs are the drivers of not only tumor initiation and heterogeneity, but also of treatment resistance, cancer recurrence, and metastasis. Patient survival depends on these CSCs, which are one cause of tumor recurrence after surgery and chemotherapy. CSCs work through a limited number of key pathways, but the detailed mechanism by which these cells function to sustain and manipulate the tumor environment is poorly understood. Therefore, target selection; an understanding of CSCs biology, the genetic and molecular profiles of CSCs, and their key signaling pathways, and; appropriate clinical trials endpoints that are designed to target CSCs will help in the development of drugs that will specifically target this small population of stem cells.

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Figure 1:
Phytochemicals regulating signaling pathways in pancreatic cancer stem cells

Table 1:
Cell surface markers and their features associated with cancer stem cells in pancreatic cancer.

Cell Surface Marker	Feature	Category of Marker	Tissue / Cell Lines/ Blood	References
CD44+CD24+	Tumorigenic and tumor-initiating cells	Surface molecule	Human pancreatic adenocarcinoma (PDAC) tissue	[75]
CD44+CD24+ESA+	Self-renewal, tumorigenicity	Surface molecule	Human PDAC tissue	[22]
CD133+ and/or CD133+CXCR4+	Higher tumorigenic and metastatic potential	Surface molecule	Human PC tissue	[76]
CD44+, CD133+	Tumorsphere-forming and tumor-initiating cells	Surface molecule	Human PDAC cells (MiaPaCa2)	[77]
c-Met ^{high} and/or c-Met ^{high} CD44+	Tumor growth and metastasis	Surface molecule	Human PDAC tissue	[78]
ABCG2+ and/or CD133	Side population phenotype (ABCG2+) and subset of stem/progenitor cells in the hematopoietic system (CD133)	Surface molecule	PDAC cells	[79]
Integrin β 3+	Driver of carcinoma stemness and drug resistance	Surface molecule	Human PDAC cells (PANC1)	[80]
ALDH1+ or High ALDH1 Activity	Tumor-initiation with enhanced tumorigenic potential	Intracellular molecule	Human PDAC tissue	[75, 81]
Low 26S Proteasome Activity	Formed spheres and underwent asymmetric division	Intracellular molecule	Human PDAC cells (PANC1, MiaPaCa2, BxPC3, and KLM1)	[82]
Delk+	Display morphologic and molecular features of gastrointestinal tuft cells	Intracellular molecule	Mouse pancreatic tissue	[83]
CD133, ABCG2, and Notch1 (Side Population Cells)	Differentiate into daughter cells and drug-resistance	Surface molecule (Others)	Human pancreatic carcinoma cells (PANC-1)	[84]

Table 2:

Role of polyphenols in chemoprevention of cancer stem cells (CSCs)

Phytochemicals	Concentration	Regulation of Cancer Stem Cells (CSCs) Markers/ Properties	Tissue / Cell Lines	References
Genistein	30 or 60 μ M	Inhibit pancreaticospheres formation and CD44 & EpCAM expression	AsPC-1-FoxM1 cells	(41, 42)
EGCG	20, 40, or 60 μ M	Inhibit spheroid formation, transcription factors (Nanog, c-Myc and Oct-4) and self-renewal capacity of pancreatic CSCs	Human pancreatic CSCs (CD133+/CD44+/CD24+/ESA+)	(48)
Quercetin and EGCG	40 μ M and 60 μ M	Inhibit self-renewal capacity of CSCs through attenuation of TCF/LEF and Gli activities		
EGCG	40 μ M	Inhibit self-renewal capacity (colony and spheroid formation, ALDH1 activity)	PDA cell lines BxPC-3 and MIA-PaCa2, human hTERT-HPNE immortalized pancreatic duct cells CRL-1097, and primary human PDA cells PaCaDD-183 from PDA patient	(49)
Quercetin (Q)	200 μ M			
Sulforaphane (SF)	200 μ M			
Epicatechin gallate (ECGC)	40 μ M			
Green tea extract (GTC)	40 μ M			
SF+GTC + Q	200 μ M + 40 μ M + 200 μ M			
GTC + SF	40 μ M + 200 μ M			
EGCG and phosphodiesterase-3 inhibitor	5 μ M and 2.5 μ M	Suppressed the Forkhead box O3 and CD44 axis	Human pancreatic carcinoma cells (BxPC-3 and MIA PaCa-2)	(50)
Sulforaphane	10 μ M	Sensitizes for TRAIL-induced cytotoxicity by inhibiting clonogenic growth, sphere formation, aldehyde dehydrogenase (ALDH) activity	Patient tumor cells (CD44+/CD24 ^{low})	(51)
Sulforaphane + Sorafenib	10 μ M/L and 20 μ M/L	Eradicated SO-induced NF- κ B binding by inhibiting clonogenicity, spheroid formation, ALDH1 activity	Human pancreatic carcinoma cells (BxPC-3 and MIA PaCa-2)	(52)
Sulforaphane + anti-cancer drugs	5 μ M/L	Inhibition of ALDH1, NOTCH-1, and c-Rel expression	Human pancreatic carcinoma cells (MIA PaCa-2)	(53)
Sulforaphane	5 or 10 μ M	Inhibited self-renewal capacity of pancreatic CSCs	CSCs (CD44 ⁺ CD24 ⁺ ESA ⁺) derived from human pancreatic cancer cells (MIA-PACA-2, PANC-1, AsPC-1, and Bx-PC-3)	(54)
Sulforaphane + Quercetin	5 or 10 μ M + 20 μ M	Synergistic inhibited effects on self-renewal capacity of pancreatic CSCs		
Sulforaphane	5–20 μ M	Inhibited self-renewal capacity of pancreatic CSCs and pluripotency-maintaining TF Nanog and Oct-4	Human pancreatic cancer cells (ASPC1 and PANC1)	(55)
Sulforaphane	20 mg/kg	Inhibited tumor growth and TF Nanog and Oct-4	Orthotopically implanted primary pancreatic CSCs in of NOD/SCID/IL2R gamma mice	(56)

Phytochemicals	Concentration	Regulation of Cancer Stem Cells (CSCs) Markers/ Properties	Tissue / Cell Lines	References
Resveratrol	10 or 20 μ M	Inhibits pluripotency maintaining factors (Nanog, Sox-2, c-Myc and Oct-4), drug resistance gene ABCG2 in CSCs	Human pancreatic CSCs (CD 133 ⁺ CD44 ⁺ CD24 ⁺ ESA ⁺)	(69)
Quercetin	100, 200, or 400 μ M	Inhibit ALDH1 activity	Human pancreatic carcinoma cells (BxPC-3 and MIA PaCa-2)	(70)
Quercetin + Sulforaphane	200 μ M+ 10 μ M			
Quercetin	1–100 μ M	Inhibit self-renewal capacity	Human pancreatic carcinoma cells (ASPC-1, BxPC-3, PANC-1, SW1990, and HPAC)	(73)