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Abstract: Pancreatic cancer (PC) is a multigenic stromal disease with a high mortality rate. Gemcitabine is a widely prescribed drug for conventional chemotherapies. However, the usage of gemcitabine has been limited due to the resistance developed in tumor cells. Combining gemcitabine with other drugs such as platinum, celecoxib, erlotinib, and bevacizumab is found effective. However, these combination regimens were found to have toxic side effects and lead to poor survival due to activation of hypoxia inducible factor-1 alpha and nuclear factor kappa-B. Transcription factors also play a crucial role in resistance and tumor recurrence. Therefore, researchers are now focused on investigating novel drugs to reduce tumor recurrence and metastasis without toxic side effects. The current review discussed the gemcitabine structure, metabolism and mechanism of action on PC growth, resistance, and signaling.

Keywords: Pancreatic cancer, Resveratrol, Gemcitabine, Resistance, Signaling, Metastasis

1. Introduction

Pancreatic cancer (PC) is one among the most lethal diseases in the U.S. and worldwide. As per estimations by the American Cancer Society, nearly 57,600 new cases were diagnosed with 47,050 deaths recorded in the U.S. for the year 2020, making it the third most lethal cancer after colorectal cancer and lung cancers⁵¹. PC ranks as the seventh most lethal cancer in both men and women, per GLOBOCAN estimations⁵３. The survival rate is only for 5 years, as 75–88% of patients were diagnosed with PC only already had advanced or metastatic cancers⁵¹,⁵３. The advances in pre-operative management, diagnosis, and therapies include radio- and chemo-therapies were made relevant to treat advanced stage of PC but are with only modest outcome. Consequently, advanced screening, diagnosis, and therapeutic strategies for PC patient are required for the researchers and clinicians to get better impact.

The evidence that regular aggressive treatments increase the lifespan of patients with PC is limited. The heterogeneity of PC negatively impacts response to therapy. The array of therapies includes surgery, chemoradiotherapy, immunotherapy, medications, hormonal therapy, and nutritional therapy. At present, PC can only be effectively controlled by few chemotherapy drugs. Gemcitabine, a widely studied cytotoxic drug for PC since 1997, is suggested as first-line chemotherapy for PC patients⁴, ₂, ₂-difluoro-2-deoxycytidine (Gemcitabine) is an analogue of pyrimidine nucleoside⁵₁. It was approved by U.S. Food and Drug Administration (FDA) in 1995 as an anti-neoplastic drug for solid type cancers,
including those of pancreas, lung, breast, and ovary as well as sarcoma and cholangiocarcinoma. The cellular toxic function of gemcitabine is associated with suppressing the ribonucleotide reductase (RNRs) to terminate DNA replication, thereby disrupting DNA synthesis and interrupting progression of cell cycle. Compared with the other nucleoside analogs, gemcitabine has unique pharmacokinetics. However, as it is a broadly used drug, almost all PC patients eventually develop resistance to this drug. Numerous cellular pathways, enzymes of nucleotide metabolism, and various transcriptional factors have been associated with sensitivity as well as resistance to gemcitabine. At present, combination therapy with gemcitabine has shown promising results but no significant improvement in survival rates. So far, gemcitabine with erlotinib (tyrosine kinase inhibitor), irinotecan (topoisomerase inhibitor), cisplatin, and oxaliplatin (platinum agent) is FDA-approved combination therapies. But unfortunately, they have a modest benefit of <1 year of the overall survival of PC patients. Although gemcitabine either used alone or in combination regimens has become a regular drug for the treatment of PC, the progression of free survival interval range from weeks to months. Due to PC’s propensity to invade, metastasize, recur, and develop drug resistance, overcoming the gemcitabine resistance has become a great issue.

2. Gemcitabine

Gemcitabine is a first-line drug for the treatment of PC, as it has demonstrated increased efficacy when compared with 5-fluorouracil (5-FU). The combination regimen of gemcitabine and nab-paclitaxel was found to be effective in treating PC. Although these therapeutic drugs are found effective for advanced metastatic stages, patients still develop chemoresistance and toxic side effects, which limit the effectiveness of chemotherapy. A varied number of aberrant transcription factors are more or less involved in the development of chemoresistance against chemotherapeutic drugs like gemcitabine. For instance, hypoxia inducible factor-1 alpha (HIF-1α) is associated with chemo- and radio-therapy resistance in PC. The ATP-binding cassette super-family G member 2 (ABCG2) proteins are multidrug resistance inducing pump proteins. In PC, HIF-1α nuclear translocation is enhanced by the hypoxia-induced ERK pathway that further binds to hypoxia responsive elements (HRE) of ABCG2 and promote drug resistance.

While heat shock protein 90 (HSP90) induces the transcription of HIF-1α, knocking down of HSP90 reverses the activation of HIF-1α to induce PC resistance to radio- and chemo-therapy. A study also showed that gemcitabine treatment induced the activation of nuclear factor kappa B (NF-κB) and HIF-1α. These nuclear transcription factors contribute to gemcitabine resistance through reactive oxygen species-mediated activation of Akt and ERK1/2 signaling pathways, upregulating expression of chemokine receptor 4 (CXCR4). Moreover, HIF-1α with signal transducer and activator of transcription 3 (STAT3) showed a negative feedback with interleukin 37 (IL-37) expression, as it inhibits the expression of HIF-1α through the inhibition of STAT3. The downregulation of IL-37 is detected in PC cells and is interlinked with inducing migration and developing resistance against gemcitabine. HIF-1α attenuated the transcription of IL-37 through binding at its promoter HRE, miR-301a induces resistance against gemcitabine under hypoxia conditions through HIF-1α enhancement as well as accumulation of HIF-1α with the inhibition of tumor protein p36 (TAp63), as it plays a crucial role in downregulating HIF-1α expression. Thus, sensitizing the tumor-promoting or -inhibiting proteins would enhance the efficacy of the chemotherapy drug. The combination therapy with other chemodrugs was always found effective. Therefore, combining the chemotherapy drugs with phytochemicals is highly encouraged as it sensitizes the PC cells to gemcitabine and enhances efficacy of the drug. For instance, resveratrol in combination with gemcitabine synergistically downregulates the expression of vascular endothelial growth factor B (VEGF-B) to decrease the phosphorylated levels of Bcl-2-associated X protein (Bax) and glycogen synthase kinase 3 beta (GSK3β). Thus, resveratrol can efficiently and synergistically enhance the efficacy of chemotherapy drugs by inhibiting the tumor-promoting factors more effectively.

2.1. Chemical structure of gemcitabine

Gemcitabine (dFdC, C9H12F2N3O4, molecular weight: 263.2 g/mol), is a nucleoside analog and one of the most promising cytotoxic drugs. Its anti-neoplastic activity depends on various inhibitory functions of DNA synthesis and cell cycle suppression. As compared with cytosine arabinoside (Ara-C; pyrimidine analogue), gemcitabine exhibits an array of unique functions and a narrow range of activity, which is associated with its metabolism, pharmacology at cellular level, and mode of action. The fluorine subunits on the second spot of the furanose ring of gemcitabine makes up the active site.

2.2. Transport of gemcitabine in PC cells

As the hydrophilic nature of gemcitabine makes diffusion across the cellular membrane difficult, effective movement into cells requires a specialized integral transport. In humans, two nucleoside transporter families exist: the human equilibrative nucleoside transporter (hENT) and the human concentrative nucleoside transporter (hCNT), which are differentiated by the SLC29 and SLC28 gene families. Various nucleoside analog, including gemcitabine, utilize these transporters to enter cells.
2.3. hCNT

It is a nucleoside drug import pump encoded by the SLC28 gene family, and uses the inwardly directed sodium gradient through ubiquitous sodium-potassium ATPase pump to transport the substances into cells\[29\]. The SLC28 family consists of three distinct proteins: CNT-1, CNT-2, and CNT-3\[30\]. Each of which has different affinity and specificity. While CNT-1 mainly transports adenosine and pyrimidine nucleosides, such as gemcitabine, CNT-2 transports uridine and purines, and CNT-3 transports both pyrimidines and purines\[31,32\].

2.4. hENT

It is encoded by a SLC29 gene family and consists of four different hENTs encoded by four different genes\[33\]. The hENTs facilitate the transport of nucleosides into the cells using concentration gradients. Both hENT1 and hENT2 are ubiquitous in plasma membranes; they facilitate transport of both purines and pyrimidine, and hENT2 transports nucleobases as well\[33\]. The hENT3 transporter is broadly circulated and moves both pyrimidines and purines, but has mostly intracellular functions with slight apparent significance on nucleoside transport across the plasma membrane (e.g., mitochondrial and lysosomal membranes)\[34-36\]. hENT4 also transports monoamines and adenosine in the heart and brain\[37\].

Gemcitabine transported into cells with hCNT1, hCNT3, hENT1, and with decreased affinity with hENT2\[38\]. Therefore, these membrane nucleoside transporters show a key role in the clinical efficiency of gemcitabine\[39\]. Clinical studies and in vitro studies have confirmed that defects in these nucleoside transporters are associated with gemcitabine resistance and decreased survival\[40-43\]. As an example, in PC patients with elevated hENT1 protein levels, gemcitabine therapy resulted in increased survival. Research by Santini et al. suggests that hENT1 acts as prognostic biomarker for gemcitabine treatment, as patients with enhanced hENT1 expression exhibited improved survival\[41\]. Similar conclusions can be drawn from various clinical studies that show how deficiencies in hENT1 correlate with resistance to gemcitabine-based therapy\[42,44-46\]. Nevertheless, cells can become sensitized to gemcitabine when transfection with hCNT3 gene through sonoporation ultrasound and microtubule method, as hCNT3 greatly enhances the gemcitabine uptake\[47\].

3. Metabolism of gemcitabine

Once gemcitabine reaches the cell, it is phosphorylated in the cytoplasm by the rate limiting enzyme deoxycytidine kinase (dCK) into gemcitabine monophosphate (dFdCMP). It is then phosphorylated by uridine monophosphate-cytidine monophosphate (UMP-CMP) kinase to produce gemcitabine diphosphate (dFdCDP) and gemcitabine triphosphate (dFdCTP)\[48,49\]. These active metabolites associated with DNA replication inhibition. dFdCTP competes with deoxycytidine triphosphate (dCTP) for integration into the DNA chain. After dFdCTP is attached to the DNA strand, it inhibits the polymerase, and two phosphates are cutoff to eliminate dFdCMP from the strand\[50\]. This allows for the addition of one nucleotide phosphate to the DNA strand before DNA replication termination can occur\[8,9\]. This unique method of blocking the DNA synthesis by dFdCMP is termed as “masked chain termination.” These molecular actions are important to gemcitabine-induced programmed death. On the other hand, dFdCDP suppresses the RNRs that changes cytidine diphosphate (CDP) to deoxycytidine diphosphate (dCDP), to reduce dCTP levels and facilitate integration of dFdCTP into DNA\[50\]. Several preclinical tumor models demonstrate that increased dFdCTP intracellular accumulation and integration of DNA are involved with high gemcitabine sensitivity, as it inhibits the de novo synthesis\[51\]. Moreover, clonogenic survival tests revealed that enhanced gemcitabine concentrations led to reduced cell viability, thereby indicating a longer period of time for intracellular release of active metabolites of gemcitabine\[9\]. Various factors, such as (i) dosing time, (ii) hENT1 cell transport, (iii) dCK phosphorylation, (iv) single nucleotide polymorphisms (SNPs) in cytidine deaminase (CDA) and dCK, and (v) CDA degradation, influence the cytotoxicity of gemcitabine and intracellular accumulation of active metabolites.

4. Mechanism of gemcitabine

Gemcitabine’s mechanism of action involves DNA synthesis inhibition. Once dFdCTP has been incorporated to DNA, polymerase enzyme incorporates a single deoxynucleotide, which inhibits the chain elongation. Thus, gemcitabine appears at the non-terminal position and stops the DNA polymerase (Figure 2). This unique mechanism of incorporation is called masked chain termination. It is

![Figure 1. Chemical structures of gemcitabine. Gemcitabine is a nucleoside analog. The fluorine subunits on the second position of the furanose ring of gemcitabine makes up the active site.](image-url)
also suppresses gemcitabine removal by inhibiting DNA proofreading enzymes.[52]

Another notable mode of action of gemcitabine is self-potentiation, which describes how drug metabolites increase the cytotoxicity of the drugs through suppression of enzymes associated with the deoxynucleotide metabolism through self-potentiation. Deoxycytidylate deaminase is directly suppressed with dFdCTP and indirectly with dFdCDP.[50] (Figure 2). RNRs catalyzes the synthesis of ribonucleotides. As RNRs allow for rapidly dividing cells, they are a potential therapeutic target for anti-viral and anti-cancer treatments.[53] The function of dCK is mainly regulated by the dCTP, which reduces the deoxynucleoside triphosphate (dNTP) pool to stimulate phosphorylation of dFDC. Thus, with an increased ratio of dCTP and dFdCTP, dFdCTP can be more readily incorporated into DNA.[54] Gemcitabine triggers p38-MAPK to stimulate programmed cell death in the occurrence of cellular stress.[55] Kopper et al.[56] studies suggest that MK2, a p38-MAPK effector, is essential for gemcitabine-induced apoptosis.

5. Signaling pathways regulating gemcitabine resistance and mechanism

Genesis of signaling cascades is a result of various receptors and stimuli. Inside a cell, multiple signaling pathways make up an interconnecting network in formation of the complex by the signaling cascades network. Mostly, chemotherapy failure is a result of intrinsic or acquired resistance and subsequent tumor reoccurrence. However, the precise mechanism of the chemoresistance is fragmentary. A number of genes influence chemoresistance, including the signaling cascades p53, PI3K/Akt, epidermal growth factor receptor (EGFR), NF-κB, RAS/RAF/MAPK, p16/CDKN2A, SMAD4, Notch, and sonic Hedgehog (Hh) pathways. These muted pathways either directly or indirectly impact pancreatic tumor chemo sensitivity.[57]

5.1. RAS/RAF/MAPK pathway

Induction of cell death through caspases can occur by mediated with the RAS/RAF/MAPK pathway in several ways. Therefore, aberrant activation of this pathway contributes to tumor growth, resistance, and development. The cytotoxic function of gemcitabine is mainly associated with the induction of p38, which results in MAPK caspase-mediated apoptosis. On the other hand, resistance to the gemcitabine increases survival pathway activity, leading to tumor development.[58,59]

Fructose-1,6-bisphosphatase-1 (FBP-1)-derived peptide inhibitor, a negative modulator of the MAPK pathway, enhances gemcitabine sensitivity in pancreatic ductal adenocarcinoma by inhibition of the ERK activation.[60] Thymoquinone, which is an inhibitor of the Akt/mTOR/pS6K pathway, increases cisplatin and oxaliplatin sensitivity in PC as well as gemcitabine sensitivity.[61] Src tyrosine kinase pathway in PC is activated by Kras. This amplification loop of Src/Erb2 stimulates Akt and provides

Figure 2. Mechanism of gemcitabine action. Gemcitabine acts as an anticancer agent a series of phosphorylation’s in PC cells. Gemcitabine forms dFdCTP by three kinase enzymes, dCK, dFdCMP, and dFdCDP. dFdCTP is an important active metabolite that exhibits the anticancer effects by incorporating with the DNA. Suppressing RNRs also reduces the intracellular dNTPs, thereby resulting in enhancement of the activity of dCKs and increases the amount of gemcitabine to its active metabolites. dCK, deoxycytidine kinase; dFdCMP, gemcitabine diphosphate; dFdCTP, gemcitabine triphosphate; dCTP, deoxycytidine triphosphate; NMPK, nucleoside monophosphate kinase; NDPK, nucleoside diphosphate kinase; RNR, ribonucleotide reductase; hENT, human equilibrative nucleoside transporter; and hCNT, human concentrative nucleoside transporter.
resistance to gemcitabine. As practically documented, most acquired gemcitabine resistance is attributable to the Kras over-activity. Overamplified Kras promotes carcinogenesis and resistance to gemcitabine\textsuperscript{62,63}. In several cancers, epithelial–mesenchymal transition (EMT) is associated with the various functions such as chemotherapy resistance and metastasis. For example, an Akt target is stabilized by GSK3β\textsuperscript{64}. Zidovudine (a thymidine analogue) enhances gemcitabine efficacy by suppressing the EMT and modifying the Akt/GSK3β pathway\textsuperscript{65}. In addition, zidovudine increases the equilibrative nucleoside transporter 1 (ENT1) function, permitting the drug entry into the cell. This study suggested that gemcitabine combination could be a new treatment the PC patients\textsuperscript{66}.

5.2. p53 pathway
Tumor suppressor p53 plays a significant role in activating the DNA damage and cellular responses to genetic abnormalities. Mutated Tp53 is associated with the gain-of-function activity, including hyperproliferation, genomic instability, and chemoresistance\textsuperscript{67}. Studies revealed that mutated p53 promotes cell-cycle dysregulation, alters the apoptosis signaling, and increases learned resistance to gemcitabine in PC\textsuperscript{68}. In fact, mutated p53 induces chemoresistance by enhancing cyclin-dependent kinase 1 (CDK1) and CCNB1 expression, leading to hyperproliferation. Nevertheless, immediate treatment of PC cells with gemcitabine combined with RITA (reactivating p53 and inducing tumor apoptosis) and CP-31398 abates the tumor growth by G1 phase arrest and enhances the gemcitabine sensitivity by inducing apoptosis\textsuperscript{69}.

5.3. Notch signaling pathway
Notch signaling pathway activity in PC is correlated with resistance to conventional drug gemcitabine. Recently, a research explained that upregulated Notch signaling pathway develops gemcitabine insensitivity in PC cells. Furthermore, inhibition of the Notch signaling by thymoquinone enhances the gemcitabine efficacy in PC cells\textsuperscript{70}. Moreover, overexpression of Notch signaling pathway enhances the cancer stem cell (CSC) activity, which leads to resistance to gemcitabine and progression of metastasis in PC cells\textsuperscript{71}. Activation of Notch associated with gemcitabine resistance to PC cells by regulating the EMT\textsuperscript{72,73}. This signaling plays an essential function in resistance to conventional drugs, as they affect CSCs and EMT.

5.4. TGF-β signaling
TGF-β is associated with EMT phenotype and resistance to gemcitabine in PC\textsuperscript{74}. Upregulation of TGF-β expression identified in many cancers, including those of pancreas, prostate and lung as well as squamous cell cancers\textsuperscript{75,76}. Furthermore, barbamine improves the effectiveness of gemcitabine in PC cells by suppressing the tumor proliferation and induction of apoptosis through activating the TGF-β/Smad signaling cascade\textsuperscript{77}. Recently, research showed that small interfering RNA (siRNA)-mediated SET binding factor 2 (SBF2) silencing can significantly suppress proliferation and induce apoptosis of PC cells by downregulating TGF-β/Smad pathways\textsuperscript{78}.

5.5. EGFR pathway
EGFR is essential for several biological processes such as cell-cycle progression, migration, and adhesion. However, aberration in EGFR pathway is concerned in cancer progression and drug resistance\textsuperscript{79}. Normal activation of EGFR by ligands induces auto-phosphorylation in tyrosine molecules positioned on the intracellular domains of the receptor. The stimulated tyrosine kinase in turn phosphorylates and triggers intracellular signaling cascades including Ras-MPK and PI3K/Akt. The suppression of tyrosine phosphorylation leads decreased proliferation and angiogenesis in tumor cells. Although preclinical studies demonstrated that EGFR stimulates pancreatic tumorigenesis, a study by Moore et al. revealed that gemcitabine improves survival in advanced pancreatic patients by adding the EGFR-targeting erlotinib\textsuperscript{79}. Further, another study revealed that inhibition of hexosamine biosynthetic pathway (HBP) enhances the gemcitabine sensitivity in PC cells through unfolded protein response (UPR) and regulation of the EGFR-Akt pathway\textsuperscript{80}. However, this disruption stimulates a cascade of events that impacts the cellular redox homeostasis and glycan synthesis by HBP pathway, leading to the overall changes in protein expression, glycation, and functional effects. This protein modification induces tumor resistance cells and secreted exosomes intricately involved in the decreased cell proliferation and boosted tumor cell chemosensitivity. Downstream pathways and proteins of EGFR, such as MAPK pathway, AKT-mTOR pathway, and redox enzymes, were reduced in response to dysregulation of glutamine metabolic pathway in PC\textsuperscript{81}. Based on the data, the studies concluded that EGFR is an effective target that could help improve gemcitabine efficacy.

5.6. HSPs
They are chaperon proteins that regulate cellular responses to various stressors. Some HSPs have multifunctional cytoprotection activity against anti-inflammatory and antioxidant actions\textsuperscript{82}. Some HSPs are act as activators for gemcitabine-induced cell death process. There are various reports describing HSP90, HSP70, and HSP27 overexpressed in several cancers, including PC\textsuperscript{83}. Overexpression of HSP27 in PC cells induces gemcitabine resistance, while treating with the KNK437 and AHCC (active hexose correlated compound) enhances the gemcitabine cytotoxic effect by downregulating the HSP-27\textsuperscript{84}. Therefore, HSPs play a significant role in association to the gemcitabine resistance in PC cells.
5.7. Tumor microenvironment

PC cells produce different types of CXC chemokines into the tumor environment: CXC ligand (CXCL)-1, CXCL-2, CXCL-5, CXCL-8, CXCL-10, CXCL-12, and CXCL-14. PC cells typically produce chemokine from the NF-κB signaling cascade. These are constitutively expressed in most of the PC patients. CXCL-12 and CXC chemokine receptor (CXCR)-4 are highly active in tumor stroma crosstalk. Studies revealed that treatment of PC cells upregulates CXCL-12 to induce ERK and Akt signaling cascade activation and promote resistance to gemcitabine. In contrast, suppression of CXCR-4 increases the gemcitabine-induced cell death[89]. PC cells upregulate CXCLs/CXCR-2 axis to stimulate various signaling cascades, including p38/ERK, PI3K, and JAK, to modulate cell migration and survival. In addition, the CXCR-2 receptor for IL-8 plays a significant role in recruiting the neutrophils at the inflammatory sites[85].

Another frequently debated pathway in tumor microenvironment of PC is the Hh pathway, which is overexpressed throughout PC oncogenic development[84]. Hh pathway is a significant cascade for morphogenesis during embryonic stages that is continuously reactive in different types of cancers[87]. Inhibition of the Hh pathway enhances the intra-tumoral vascular volume, which improves sensitization to gemcitabine. Similarly, in vitro studies suggest that gemcitabine-resistant cells can be sensitized with inhibition of Hh signaling cascade combination through histone deacetylase[88]. This enhances the survival rate by inhibiting the Hh signaling pathway[89]. Therefore, gemcitabine combined treatment with Hh signaling cascade inhibitors may overcome chemoresistance in PC.

In PC, tumor associated macrophages (TAMs) are associated with resistance to gemcitabine, as activation of cytidine deaminase induces the caspase-3 pathway to metabolize gemcitabine into inactive form[90]. In addition, TAMs were identified to inhibit antitumor immune responses. Conversely, TAM chemotaxis to the PC stroma was suppressed by C-C chemokine receptor type 2 (CCR2)/colony-stimulating factor 1 (CSF1) or CCR2[90,92]. Data suggest that inhibiting tumor-stromal interaction may enhance the drug efficacy in PC.

5.8. NF-κB pathway

NF-κB is found aberrantly activated in PC and contributes to proliferation, migration, metastasis, and EMT[93]. The NF-κB activation also promotes the secretion of VEGF and CXCL-8[88]. Similarly, STAT3 also induces lymphatic metastasis through the activation of VEGF-C in PC[95]. They also induce the expression of other angiogenic inducing factors, such as IL-8, as well as EMT-related genes, such as matrix metalloproteinase (MMP)-2 and MMP-9, which are essential for metastasis[96].

In PC, NF-κB induces chemoresistance to gemcitabine[97]. Blocking of the p65 subunit decreases the NF-κB pathway, and thus suppresses the cyclin-D1, Bcl-2, VEGF, and caspase-3 stimulation. Gemcitabine could potentially combine with p65 siRNA to suppress growth of PC[97,98]. Various pathways that suppress the NF-κB pathway reduce gemcitabine resistance in PC.

5.9. HIF-1α pathway

A number of studies confirmed that HIF-1α is associated with gemcitabine resistance through signaling pathways of DNA damage, autophagy, p53, apoptosis, and drug efflux. Hypoxia decreases the pH levels and forms acidic microenvironment, which leads to multidrug resistance. This includes reduced amount of drug caused by increased functions of multidrug transporter p-glycoprotein, decreased apoptosis, ion trapping, and genetic mutations[99].

In normal oxygen conditions, HIF-1α has half-life in cytoplasm of <5 min. However, in hypoxic conditions, HIF-1α stabilizes and escapes degradation to enter the nucleus. In nucleus, HIF-1α dimerizes with HIF-1β to form the transcriptional factor HIF-1[100], which combines with HREs to activate the transcription of various O2-dependent genes[101]. Oxygen deficiency creates stressful environment to promote apoptosis in hypoxic regions[102].

Tumor cells can survive in hypoxic environment, and hypoxia itself can stimulate adaptive cellular responses involved in tumor progression. Various HIF-1α-associated biological activities are helpful for tumor development, including induction of angiogenesis and apoptosis, alterations in metabolism, stimulation of migration to escape hostile regions, and resistance to therapy[101,104]. HIF-1α, a transcriptional factor, not only plays a significant role in PC, but is also associated with various cancers. Hence, understanding the relation and its function in PC progression and metastasis can aid in the future in find out therapies. As mentioned above, PCs are highly aggressive tumors with a poor 5-year survival rate. The late-stage diagnosis and surgical removal of primary PC tumors often ends in disease reoccurrence. Further, the radiation and first-line gemcitabine-based chemotherapy of advanced and metastatic PC patients show improved survival rate.

Hypoxia-activated growth factors play a significant role in PC proliferation and survival. Assuming that cell proliferation is associated with synthesis of nucleic acid, protein, and lipid, it is crucial to reorganize metabolic functions to support proliferation of inactive cells[105]. For example, when oxygen level decreases, the rate of glycolysis increases to maintain energy production. Similarly, enhanced rate of glycolysis is an important element for upregulated HIF-1α function. Initially, it has been testified that glioma cells are classified by a positive feedback loop associated HIF-1α activation, lactic acid, and pyruvic acid[106-108]. This research demonstrated that with blocking of HIF-1α, proliferating cell nuclear antigen (PCNA) expression levels decrease and the contributory
effect of HIF-1α on cell proliferation vanished. In vitro studies recommended that during hypoxic situations, pG1 cells grow at a faster rate. On inhibiting the HIF-1 expression by siRNA, pG2 cells became more sensitive to hypoxic situation, and demonstrated slow growth under normal and hypoxic milieu[109]. A study by Wie et al. described that HIF-1 under hypoxic situation promotes PC cell proliferation. In vivo studies explained that pG2 cells inhibited by HIF-1 could not generate tumor under hypoxic microenvironment due to the reduced resistance to hypoxia. Under normoxia, tumors resulting from pG1 cells outnumbered those acquired from pG2 cells, indicating a unique feature of HIF-1 on PC proliferation under hypoxia and normoxia[110].

HIF-1α also plays a crucial role in regulating the expression of VEGF[111]. HIF-1α is the transcription factor commonly upregulated under hypoxic conditions, and then translocates into nucleus through mucin 1 (MUC1). They together maintain crosstalk to promote glucose metabolism and impart resistance in PC against gemcitabine[112]. Pyruvate kinase muscle isozyme (PKM2) in PC upregulates the expression of HIF-1α and NF-κB-p65 to induce VEGF-A expression[113]. Furthermore, HIF-1α also maintains crosstalk with STAT3 to mediate angiogenesis through VEGF expression and the autocrine IL-6/HIF-1α/STAT3 loop[114]. HSP90 chaperon in PC also promotes angiogenesis via the upregulation of STAT3 and HIF-1α. In addition to VEGF secretion, HIF-1α promotes the platelet derived growth factor A (PDGF-A) expression[115], while MMP-2 and MMP-9[116] play crucial role in promoting angiogenesis and metastasis. HIF-1α is the downstream molecule of mTOR pathway regulated by glucagon like peptide-1 (GLP-1) receptor[117]. Moreover, the hyperglycemia promotes the overexpression of HIF-1α to upregulate MMP-9, induce hypoxic tumor microenvironment and promote PC progression. The previous studies have demonstrated that HIF-1α mediates EMT, angiogenesis, and metastasis in PC under hypoxia. In addition, hyperglycemia under hypoxic conditions promotes HIF-1α expression in PC cells to induce the expression of its downstream molecule, VEGF[118]. Moreover, HIF-1α inhibits the expression of E-cadherin by inducing the complex of metastasis associated protein 2/HDAC1 complex[119]. The complex binds to the promoter of E-cadherin to inhibit it and promote EMT. E-cadherin plays a crucial role in epithelial cell polarity and maintains cell-cell adhesion[120]. In addition, HIF-1α also promotes Twist protein and zeste homolog 2 (EZH2) expression, to inhibit the transcription of E-cadherin via binding to its promoter[121]. Thus, HIF-1α serves as an EMT promoter to induce metastasis in PC cell. HIF-1α also intervenes with other proteins, including lncRNA-BX111887, to promote activation of zinc finger E-box binding protein 1 and thereby EMT (ZEB1)[122], miRNA-142 downregulation is positively correlated with poor prognosis in PC patients, as its downregulation induces HIF-1α expression and promotes EMT in PC cells[123]. HIF-1α also maintains crosstalk with the Wnt/β-catenin signaling cascades that promote the transcription of HIF-1α. HIF-2α then complexes with β-catenin to enhance β-catenin transcription. However, there is no competition interaction detected between HIF-1α/β-catenin/HIF-2α but the interaction between HIF-2α/β-catenin promotes tumor invasion and angiogenesis in PC[124].

6. Conclusion

PC exhibits a delayed response to the chemotherapy. The chances of recurrence are high and survival rate is very low due to the activation of multiple transcriptional proteins and signaling cascades associated with inflammation, abnormal cell cycle activity, metabolism, and loss of apoptotic activity. Gemcitabine, which is a FDA-approved drug, is currently used to treat patients diagnosed with advanced stages of PC. However, gemcitabine exhibits poor prognosis due to the resistance developed during the initial treatment. Thus, identifying effective therapies and preventive agents is vital towards successful PC treatment. Since diet is a major contributing factor for PC, it would be beneficial to examine dietary bioactive compounds or synthetic small molecules. Due to their non-toxic and multitriggered nature, dietary compounds could potentially play a beneficial role in improving efficiency of gemcitabine.

Conflict of interest

The authors declare no conflict of interest.

Author contributions

G.S. and G.P.N. conceived the idea of this review and wrote the paper. P.S., A.A., and G.P.N. reviewed and edited drafts of the paper. All authors have read and agree to the published version of the manuscript.

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