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Chapter

Advance in Pancreatic Cancer Diagnosis and Therapy

Xiaojie Cai, Jie Gao, Yanfang Liu, Ming Wang, Qiulian Ma, Aihua Gong, Dongqing Wang and Haitao Zhu

Abstract

Pancreatic carcinoma is the fourth leading cause of cancer death in the word wild. Although the advance in treatment this disease, the 5-years survival rate is still rather low. In the recent year, many new therapy and treatment avenues have been developed for pancreatic cancer. In this chapter, we mainly focus on the following aspect: 1) the treatment modality in pancreatic cancer, including chemotherapy, radiotherapy, and immunotherapy; 2) the mechanism of pancreatic cancer treatment resistance, especially in cancer stem cells and tumor microenvironment; 3) the diagnosis tools in pancreatic cancer, including serum markers, imaging methods and endoscopic ultrasonography. Novel molecular probes based on the nanotechnology in the diagnosis of pancreatic cancer are also discussed.

Keywords: pancreatic carcinoma, immunotherapy, cancer stem cell, tumor microenvironment, nano-medicine

1. Introduction

Pancreatic cancer is currently the fourth leading cause of cancer-related death and is predicted to be the most common cause of cancer mortality by 2030 [1]. Despite advances in the treatment of pancreatic cancer, prognosis remains extremely poor with 5-year survival of only 8% [2]. The low survival rate is attributed to several factors, such as asymptomatic until the disease develops to an advanced stage, early and extensive metastasis, and high resistance to treatment. Therefore, precision diagnosis and effective treatment is a critical clinical issue.

Currently, commonly employed treatments for pancreatic cancer include surgery, chemotherapy, and radiation therapy. Surgical resection is regarded as the only treatment for curing pancreatic cancer. However, only 15% of pancreatic cancer patients present with disease that are resectable upfront. Chemotherapy is the mainstream treatment for local, advanced and metastatic pancreatic cancer [3]. Among the traditional treatment, chemotherapy is the most advanced modality, especially the target therapy. The role of radiotherapy in pancreatic cancer is still controversial. Although the clinical trial results were disappointing, immunotherapy is the still greatly investigated approaches in pancreatic cancer. The deeper investigation of treatment resistance mechanism and novelty modality development is urgently needed.

2. Treatment modality of pancreatic cancer

At present, commonly employed treatment for pancreatic cancer include surgery, chemotherapy, and radiotherapy. The treatment options depend on the stage of pancreatic cancer. Some emerging therapeutic technologies have yet to mature, such as molecular targeted therapy and immunotherapy.

2.1 Surgical therapy

Surgical resection is regarded as the only treatment for cure and can result in significantly longer survival of pancreatic cancer. According to the diseased localization and extension, pancreatic cancer is divided into resectable, borderline resectable, or locally advanced. Resectable cases account for 15% of pancreatic cancer patients and this subpopulation is the only potential for cure. However, 5-year survival is at best 20–25%. Borderline resectable cases account for another 5–10%. For some patients with early recurrence or not have the complications of aggressive disease and latent metastasis, neoadjuvant therapy is one alternative measures to reduce the tumor burden and obtain better local control. The proper sequence of surgical therapy and neoadjuvant therapy is the determine factor. Delivering full-dose chemotherapy preoperative therapy may be more effective than postoperative therapy because the resected tumor bed is associated with poor drug delivery. In patients with borderline resectable pancreatic cancer after effective neoadjuvant therapies, the possibility for an R0 resection is higher, and survival of patients who underwent surgical resection is better than that of those who did not [4]. Approximately 30–40% of patients have locally advanced unresectable pancreatic cancer (LAPC) in which tumor is involvement of neighboring blood vessels [5].

2.2 Chemotherapy

Chemotherapy is the mainstay treatment for advanced and metastatic pancreatic cancer. Fluorouracil and gemcitabine are the first line chemotherapy drugs. However, their clinic effective is still disappointing. In recent years, the National Comprehensive Cancer Network (NCCN) guidelines have recommended two options: one is the FOLFIRINOX regimen of four drug combination (fluorouracil + calcium folate + oxaliplatin + irinotecan), another is the AG regimen of a combination of paclitaxel and gemcitabine [6]. Although the four-drug combination scheme is effective to some extent, its toxicity and side effects are great. Considering the physical strength score of some patients, the application of this scheme is limited. The albumin paclitaxel regimen is relatively safe and has fewer adverse reactions. More and more researches recommend this regimen as the first-line treatment of pancreatic cancer in the future. The following subsets are specially suitable for the albumin paclitaxel treatment, such as neoadjuvant and salvage chemotherapy patients, postoperative adjuvant chemotherapy patients, and advanced chemotherapy patients [7].

2.3 Radiotherapy

Radiotherapy is always used as a curative treatment for localized cancer or lymph node metastasis, and as a palliative treatment in patients with widespread disease. Overall, nearly 50–60% of patients with cancer receive radiotherapy [8]. The role of radiotherapy in pancreatic cancer is controversial. Multiple clinical trials have been designed to access the role of radiology in pancreatic carcinoma

over the last 30 years and mixed results were acquired. According to the LAP-07 trial, no benefit was found to the addition of radiotherapy to gemcitabine for locally advanced pancreatic cancer [9]. The American society of radiation oncology's (ASTRO) guidelines recommended the clinical practice of radiotherapy for high-risk pancreatic cancer patients. It is recommended to conditionally undergo fractional radiotherapy or stereotactic body radiation therapy (SBRT) after chemotherapy in surgically resectable patients. The conditional provision of conventional fractional radiation is recommended in positive lymph nodes and margins were found during surgical resection. Neoadjuvant chemotherapy combined with radiotherapy is conditionally recommended after systemic chemotherapy for patients with resectable boundaries. It is recommended to conditionally concurrent chemo or radiotherapy or SBRT as salvage radiotherapy after systemic chemotherapy in locally advanced patients who are not suitable for surgery. New radiotherapy technology, such as intensity modulated radiation therapy (IMRT), SBRT, with advances in motion management, target delineation, treatment planning, and image guidance, allows for reducing treatment-related toxicities, improving control of micro-metastatic disease and dose escalation, as well as possible synergy between radiation and other therapy. Therefore, there is great potential for radiation to improve future outcomes in pancreatic cancer. [10].

2.4 Immunotherapy

Immunotherapy is a treatment that eliminates tumor cells by reactivating and enhancing the anti-tumor immune response of tumor patients. Much excitement has been generated immunotherapy in tumors that are refractory to traditional treatment, as well as resistance to traditional agents. Moreover, cancer immunotherapy has gone all the way from a promising preclinical application to a clinical reality. A variety of tumor associated antigens are high expression in pancreatic cancer, such as mucin1 (MUC-1), carcinoembryoni-cantigen (CEA), prostate stem cell antigen (PSCA), vascular endothelial growth factor (VEGF), mesoth-elin (MSLN) and K-ras mutation. Unfortunately, the use of immunotherapy alone has encountered disappointing results in clinical trials in pancreas cancer, with response rates only [10]. Immunotherapy includes the following methods:
(1) Active immunotherapy. Active immunotherapy refers to immunizing tumor-associated antigens to stimulate tumor-specific immune response of the body to eliminate tumors. Tumor associated antigens (TAAs) have been widely explored as cancer vaccines for treatment of pancreatic cancer in both mouse models and clinical trial [11]. Due to a variety of the tumor associated antigens expressing on the pancreatic cancer cells, several vaccines can be explored for the pancreatic cancer active immunotherapy, such as GVAX vaccine. K-ras gene has a high mutation rate in pancreatic cancer and K-ras vaccine has become an important target for immunotherapy of pancreatic cancer. Studies have found that the cationic nano-encapsulated K-ras peptide vaccine has a significant therapeutic effect on pancreatic cancer xenograft mice, and can significantly prolong the survival of mice [12]. In a I phase of clinical trial, following inoculated with MUC-1 peptide-loaded DC vaccine in 7 pancreatic cancer patients, the number of mature DC cells in 2 of them increased significantly and peripheral blood lymphocytes were activated and produced large amounts of IL-12p40 and IFN-γ [13]. **(2) Passive immunotherapy.** Passive immunotherapy refers to substances with immune effects are modified in vitro and then injected into human body to enhance anti-tumor immune response and eliminate tumors. At present, passive immunotherapeutic strategies used for pancreatic cancer including: 1) Antibody-mediated passive immunotherapy. Antibody-mediated passive immunotherapy involves targeting tumors using

monoclonal antibodies, antibody-drug conjugates, antibody fragments, or radioimmunotherapy conjugates to inhibit oncogenic signaling, immune suppression, or immune checkpoints [11]. Combination anti-CD40 antibody with gemcitabine showed an effective tumor inhibition. 2) Passive T cell mediated immunotherapy. Passive T cell mediated immunotherapy includes adoptive T-cell transfer and chimeric antigen receptors (CAR) T-cells therapy. **(3) Immune checkpoint blocking therapy.** Immune checkpoint blocking therapy is an immunotherapy method that can reverse the immunosuppressive signal by blocking the immunoregulatory molecules and enhance the anti-tumor immune response. Pancreatic cancer tumor cells overexpress immunosuppressive ligands, such as B7-1, B7-2 and PD-L1, which bind to surface inhibitory receptors CTL-4 and PD-1 of T cells to suppress effector T cell activity and evade immune surveillance. In 2011, the FDA approved Ipilimumab, the first humanized monoclonal antibody targeting CTL-4, for the treatment of patients with advanced melanoma. Its effect on pancreatic cancer has entered the clinical trial stage. Studies have shown that Ipilimumab can promote the proliferation of T cells and the secretion of Th1 cytokines, and enhance the killing effect of CD8+ T cells on Colo356/FG pancreatic cancer cells [14]. Two PD-1 blocking antibodies pembrolizumab and nivolumab are FDA approved for use in the treatment of metastatic non-small cell lung cancer, melanoma, renal cell cancer, and head and neck cancer [15]. In mouse model of pancreatic cancer, anti-PD-1 or PD-L1 blocking treatment promoted the generation of CD8+ T cells to tumor invasion and anti-tumor immune response. In a number of clinical trials, no objective tumor remission was observed in pancreatic cancer patients treated with anti-PD-1 or anti-PD-L1 blocking alone, suggesting that PD-1 or PD-L1 blocking alone does not have a good therapeutic effect on pancreatic cancer. **(4) CAR-T therapy.** Chimeric antigen receptor (CAR) is composed of the single chain variable fragment (ScFv) of monoclonal antibody, the hinge region and the transmembrane region of TCR receptor, and the intracellular signal transduction region in series. They form the chimeric antigen receptor by viral infection or electrical transformation on the surface of T cells. CAR-T can recognize antigens on the surface of tumor cells directly without being restricted by HLA molecules. Therefore, CAR-T has a broader application prospect in tumor immunotherapy. Target specific CAR-T cells were designed to target the highly expressed tumor-associated antigens of pancreatic cancer, making the treatment more specific. Tn-MUC-1 CAR-T: Posey et al. designed CAR-T cells targeting the Tn/STn glycopeptide phenotype on MUC-1 [16]. When CEA + C15A3 pancreatic cancer cells were transfected to mice, the cancer cells were quickly cleared and serum levels of IL-1β and IL-5 were significantly increased [17]. PSCA CAR-T: PSCA is also a tumor-associated antigen highly expressed in pancreatic cancer, and CAR-T targeting PSCA has a significant anti-tumor effect on xenograft mice of human pancreatic cancer after treatment, in which 40% of the tumors in mice have completely subsided [18]. MSLN CAR-T: Hingorani et al. designed CD8+ CAR-T cells targeting MSLN, and found that MSLN CAR-T cells could specifically kill KPC tumor cells and produce a large amount of IFN-γ in vitro. The metastasis rate dropped from 64% to 46%, and overall survival increased from 54 days to 96 days [19].

Due to unique tumor microenvironment of pancreatic cancer, both traditional treatment and single immunotherapy is not ideal. Although tumor vaccine can induce the activation of effector T cells, the activation degree is very limited and only a few effector T cells and NK cells exist in the tumor microenvironment and peripheral blood of pancreatic cancer patients. Although immune checkpoint blocking therapy can block the inhibitory effect of effector T cells, there are still many soluble immunosuppressants inhibiting effector T cells in the tumor microenvironment of pancreatic cancer. For CAR-T treatment, in addition to being

influenced by immunosuppressive factors in the tumor microenvironment, the fibrous stroma layer around pancreatic cancer cells can prevent the infiltration of CAR-T into the tumor and further its efficacy. Therefore, a deep understanding the characteristics of the immune microenvironment of pancreatic cancer and its impact on immunotherapy and/or other traditional treatment will greatly improve the treatment effect. The combination of immune checkpoint blocking therapy with radiotherapy, chemotherapy and tumor vaccines can enhance the function of tumor-specific T cells and promote lymphocyte infiltration into the tumor site. Anti-CD40 agonist can reverse the resistance of pancreatic cancer mice to PD-1 and CTLA-4 blocking antibodies, and improve the therapeutic effect of blocking antibodies. Pembrolizumab (PD-1 blocking antibody) and nivolumab (CTLA-4 blocking antibody) combined with radiotherapy can significantly prolong the survival of mice with pancreatic adenocarcinoma [20]. In order to design a reasonable immunotherapy strategy according to the characteristics of pancreatic cancer microenvironment and improve the effect of pancreatic cancer immunotherapy, we should start from the following aspects: (1) destroy the fibrous matrix layer of pancreatic cancer and increase the infiltration of effector T cells into the tumor; (2) remove excessive immunosuppressive cells such as Tregs and MDSCs, and reverse the immunosuppressive microenvironment; (3) recruit more T cells to the tumor site to enhance the anti-tumor immune response of effector T cells; (4) enhance the targeting and killing of effector T cells.

3. Treatment resistance of pancreatic cancer

Radiotherapy and chemotherapy plays a central part in curing pancreatic cancer. The major cause of treatment failure with pancreatic cancer treatment strategies mainly focus on the cancer cell itself and their localized tumor microenvironment (TME). Some of the tumor cells are already resistant to the "achievable" of anticancer treatment, termed intrinsic resistance. Other tumor cells are initially sensitive but become resistant during the course of treatment, termed acquired resistance. Regardless of the intrinsic or acquired resistance mechanisms, cancer stem cells are thought to be the major cause of tumor treatment resistance. TME refers to the internal environment in which tumor cells interact with their surrounding tissue components to form a complex and conducive to the biological behavior of tumor cells.

3.1 Tumor microenvironment

Tumor microenvironment is generally composed of three parts: (1) matrix components: extracellular matrix (ECM) and stromal cells; (2) cell components: including endothelial cells and immune cells; (3) soluble factors: including cytokines and immunoregulatory molecules [21]. The components of the tumor microenvironment are conducive to tumor proliferation, invasion, adhesion, angiogenesis and anti-radiation chemotherapy, and promote the generation of malignant tumors. Pancreatic tumor microenvironment has its own characteristics: (1) a rich of matrix components, such as pancreatic stellate cells (PSC), cancer associated fibroblasts (CAF), typeI collagen, hyaluronic acid and other extracellular matrix; (2) immune cells; (3) a large number of soluble immunosuppressive factors [22].

Pancreatic cancer cells secrete platelet-derived growth factor (PDGF), transforming growth factor beta (TGF-β), AngiotensinII and other cytokines [23]. These cytokines can activate PSCs depending on several signaling pathways, such as extracellular signal-regulated kinase (ERK), c-jun nh2-terminal kinase (JNK), p38

mitogen-activated protein kinase (P38MAPK), Janus kinase-signal transducers and activators of transcription (JAK–STAT), and phosphatidylinositol 3 kinase (PI3K) [24, 25]. The activated PSCs secrete a variety of growth factors in paracrine manner, and further promote the growth and proliferation, inhibit apoptosis and enhance their invasion ability of pancreatic cancer cells, leading to the treatment resistance [26]. CAFs are the critical part of pancreatic cancer microenvironment and function in secreting extracellular matrix proteins and participating in the formation of tumor blood vessels. CAFs also secrete interferon-gamma (IFN-γ) and tumor necrosis factor-alpha (TNF-α), which further inhibits the function and infiltration of effector T cells and induces the immunotherapy failure [27]. A large amount of extracellular matrix, including collagen, fibronectin I, III, XI and hyaluronic acid exist around the pancreatic cancer cells. Extracellular matrix creates a favorable tumor microenvironment for the growth of pancreatic cancer cells [28]. Moreover, accumulated extracellular matrix leads to the collapse and occlusion of intratumoral blood vessels, making anti-tumor drugs and immune cells fail to reach the tumor, which is useful for the chemotherapy resistance and immune escape [29].

Immune cells are rich in the TME. In pancreatic cancer, the immunity is in a state of imbalance between immune cell number and function. The number of CD4 + T cells, CD8 + T cells, NK cells and DC cells is decreased and present in an inactive or immature phenotype and state. However, CD4+ regulatory T cells (Tregs), myeloid-derived suppressor cells (MDSC), and tumor associated macrophages (TAMs) with immunosuppressive effect are active and abundant [30]. The "incapacity" state of effector cells, abundance of immunosuppressive cells and their released soluble immunosuppressive factors form the immunosuppressive microenvironment of pancreatic cancer [31].

Exist of soluble immunosuppressive factors in the microenvironment of pancreatic cancer is the important mechanism for tumor cells evading immune surveillance. (1) Transforming growth factor-β (TGF-β): TGF-β is a well-studied cytokine that is secreted by various immune cells (Tregs, MDSCs and TAMs) and tumor cells [32]. TGF-β has a dual action in cancer, as a tumor suppressor and a tumor promoter. As a tumor promoter factor, TGF-β can affect both the adaptive and innate immune systems and contributes to the evasion of immune surveillance [32]. TGF-β directly inhibits CD8 + T cell cytotoxicity [33], stimulates the generation of Tregs and contributes to exclusion of T cells from the tumor core [34]. TGF-β also inhibits NK cell proliferation and cytotoxic functions and affects myeloid cells (tumor-infiltrating macrophages and neutrophils) immunosuppressive activity [35]. In addition, TGF-β also promotes pancreatic cancer progression and metastasis depending on promoting the growth of fibroblasts and the formation of tumor extracellular matrix, and inducing tumor cells to secrete VEGF and matrix metalloproteinase 2 (MMP2) [36]. (2) IL-10: IL-10 is mainly produced by Tregs and TAM and inhibits antigen presenting cell (APC) and effector T function [36]. (3) Indoleamine-2, 3-dioxygenase (IDO): IDO is mainly produced by MDSCs and pancreatic cancer cells. Its role is to catalyze the decomposition of tryptophan necessary for the activation of effector T cells into Kynurenine, thus inhibiting the activation of effector T cells [37]. Moreover, IL-10, IL-13 and IL-23 secreted by activated fibroblasts promote the transformation of CD4 + T cells into Th2 or Th17 helper T cells, which is help to the tumor immune promoter microenvironment [38]. CCL5, CCL22 and CCL17 recruit monocytes and Tregs cells to accumulate in the tumor site, which is useful for the tumor immunosuppressive microenvironment [39].

One of the most prominent features of pancreatic cancer is featured with the asymmetry distribution of nutrients, insufficient oxygenation (hypoxia), acidic pH (acidosis), and elevated levels of reactive oxygen species (ROS). Hypoxia is one of the hallmarks of pancreatic cancer and the major drivers of tumor radio and

chemo-resistance. Hypoxia induces radio- or chemo-resistance through a variety of mechanisms. Firstly, hypoxia protects cancer cells from DNA damages [40, 41]. Secondly, hypoxia drives treatment resistance by accumulating and stabilizing hypoxia-inducible factor-1α (HIF-1α)[40, 42]. HIF-1a induces excessive secretion of proangiogenic signals, such as vascular endothelial growth factor (VEGF), and results in rapid but abnormal tumor vessel formation, which reduce the chemotherapy drug accumulation in the tumor bed. HIF-1α activation also increases the expression of key enzymes that drive the accumulation of lactate and pyruvate as well as the antioxidants glutathione and NADPH. NADPH scavenge reactive oxygen species (ROS) generated by radiation exposure to limit DNA damage [41]. Lactate up-regulates the HIF-1α pathway creating a futile cycle of radio- and immune resistance [42, 43]. In addition, radiation-induced vascular damage can enhance tumor hypoxia and trigger an immune response by increasing the production of cytokines/chemokines, thereby inducing the replenishment of immune cells. Subsequent tumor revascularisation occurs via HIF-1α dependent and independent recruitment of bone marrow-derived cells [44, 45].

3.2 Cancer stem cells (CSCs)

CSCs are a very small subset of relatively quiescent cells in the tumor that are endowed with the ability to self-renew and differentiate into non-stem daughter cells that make the bulk of tumor. Epigenetics has been implicated in many aspects of CSC biology and its role has been extensively studied [46]. Molecular determinants involved in various types of epigenetic modification, including DNA methylation, histone modification. Recent work has shown that miR-205 in combination with GEM was more efficient in reducing the proliferation of CSCs and resensitized GEM resistant pancreatic cancer cells to GEM [47].

Many signaling pathways are frequently deregulated in CSCs including Myc, Notch, Hedgehog (Hh), Wnt, FGF/FGFR, EGF/EGFR, NF-κB, MAPK, PTEN/PI3K, HER2, JAK/STAT and so on [48, 49]. Furthermore, altered cell cycle regulation can play a role in CSC quiescence, proliferation and apoptosis [50]. Cell cycle regulators are frequently lost (p53, Rb, p16/CDKN2A, CDKN1B) or amplified (CCND1, CDK4,CCNE1) in pancreatic CSCs [51]. Altered cell cycle program in pancreatic CSCs help them resist therapy-induced apoptosis [50].

Aside from intrinsic factors, extrinsic factors also contribute to CSC treatment resistance biology. Like normal SCs, CSCs reside in and rely on specialized tumor microenvironments, called niche, to maintain a balance between self-renewal and differentiation and therapy resistance. The CSC niche in pancreatic cancer is composed of a variety of stoma cells including inflammatory cells, immune cells, vascular endothelial cells, fibroblasts, smooth muscle cells, mesenchymal cells, adipocytes, nerve fibers and neural cells, together with extracellular matrix (ECM)[52]. These various components collaboratively interact with each other via networks of cytokines, chemokines and growth factors to create a hypoxia inflammatory, and immunosuppressive environment that facilitates pancreatic cancer treatment resistance [52]. The special pancreatic CSC niches include the hypoxia niche and the perivascular niche. In pancreatic cancer, hypoxia has been shown to promote the CSC expansion [53]. Oxygen plays a crucial role in generating ROS that mediate the anticancer effects of radiotherapy and chemotherapy. The low oxygen tensions in the hypoxia area of the tumor contain low levels of ROS, reducing the risk for the cells being killed [54]. In addition to physically protecting of the niches, other components of tumor microenvironment including extracellular matrix (ECM), cancer associated fibroblasts, immune cells and inflammatory cells also play a role in protecting pancreatic CSCs from both chemotherapy and other

therapies [55]. They provide CSCs with resistant signaling stimuli through surface receptors to activate other lines of defense for CSCs. Stromal cells secrete high levels of HGF which makes co-cultured human pancreatic cancer cells acquire resistance to various anticancer drugs particularly RAF inhibitors [56]. Other growth factors or cytokines including interleukin 6 (IL-6), fibroblast growth factor (FGF) and neuregulin 1 are reported to help form the so-called 'chemo-resistant niche' of CSCs by activating various survival signaling pathways [57, 58].

In addition to the niches, CSC could activate the second line of defense under treatment stress, i.e., the drug efflux mechanisms that pump the drug out of the cell are another special defense for pancreatic CSC treatment. The transmembrane proteins of the ABCT family are the main players of drug efflux and highly expressed on pancreatic CSCs [59], including multidrug resistance-associated protein 1 (MRP1 or ABCC1) and breast cancer resistance protein (BCRP or ABCG2] [59].

In case of the drug efflux has failed and the drug has invaded the cytoplasm of CSCs, high levels of drug inactivating enzymes or low expression of the drug activating enzymes would make the cells resistant to the drugs. Thymidine phosphorylase converts capecitabine into 5-fluorouracil (5-FU)[60].

Unless unrepairable DNA damage occurs, DNA repair is another main reason for radio- or chemo-resistance of pancreatic CSCs [61, 62]. It has been shown that DNA damage checkpoint and repair proteins such as the ATM, Chk1/2,p53, BRCAs and XRCC5 are aberrantly overexpressed or over-activated in CSCs but not in non-CSCs [63], and is further activated by DNA-damaging therapy such as radiation rendering delayed cell division and prolonged DNA repair time leading to resistance [64, 65]. Similar to normal stem cells, CSCs rely on specific signaling pathways for maintaining essential proliferation, survival and the balance between self-renewal and differentiation. In response to therapies, CSCs either over-activate pro-survival and anti-apoptotic signaling or down-regulate proapoptotic signaling as another mechanism of resistance to therapies [66]. For example, inhibition of NF-κB hinders the stemness of CSCs in pancreatic cancer.

Lastly, regeneration of CSCs by EMT is a likely mechanism for radio- or chemo-resistance of cancer and relapse. EMT program has been linked to the acquisition of aggressive traits and treatment resistance in CSCs [67]. A set of pleiotropic EMT transcription factors (eg. Snail1/2, Zeb1/2, Twist) together with EMT inducers (eg. TGF-β) have been proven to contribute to CSCs treatment resistance [68]. Indeed, rapid repopulation of CSCs is believed to occur in human tumors during radiotherapy [69], and redistribution of CSCs to the quiescent phase of the cell cycle makes the cells more resistant to radiotherapy.

4. Diagnosis of pancreatic cancer

Traditional methods of diagnosing early pancreatic cancer include serum markers, imaging methods and endoscopic ultrasonography. Emerging nanotechnology and advanced materials are becoming novel strategies for pancreatic cancer diagnosis. The application of multiple diagnostic methods can help to detect pancreatic cancer in the early stage, which is help to improve the survival rate.

4.1 Serological mark

During the development of pancreatic cancer, it can actively secrete certain substances, which have been preliminarily proved to be useful for the diagnosis and prognosis evaluation of pancreatic cancer. Carbohydrate antigen 19-9(CA19-9) is the most commonly used serological marker in diagnosis of pancreatic cancer [70].

The sensitivity and specificity of CA19-9 in the diagnosis of pancreatic cancer are not high, which limits its clinical application. Combination CA19-9 with CA125 significantly improve the sensitivity of pancreatic cancer detection and contribute to its early diagnosis [71]. Dj-1, a protein secreted by pancreatic adenocarcinoma cells, was found to be positive in 68.5% of pancreatic cancer samples and significantly increased in the blood sample of pancreatic cancer patients [72]. Soluble complement iC3b was found to be able to detect tumor recurrence at the early stage and more sensitivity than imaging [73]. Recent studies have also suggested that tumor-associated antigen MUC-1 specific antibody, TAB004, may be useful in the diagnosis of pancreatic cancer [74]. In addition, combination CA19-9 with both REG4 and tumor necrosis factor-a family member, APRIL, significantly improve sensitivity to the diagnosis of pancreatic cancer [74, 75]. A recent study has shown that a serum protein biomarker panel consisting of CA125, CA19-9, and laminin γC (LAMC2) significantly improve performance in detecting pancreatic cancer than single serum marker [76].

In addition to traditional serum tumor markers, some novel circulating tumor markers has made great process. MicroRNAs are a group of small non-coding RNA, consisting of 19 to 25 nucleotides, which are involved in the growth, proliferation and differentiation of pancreatic cancer. Multiple studies have confirmed that abnormally expressed serum microRNAs, such as miR-21, miR-196a and miR-155, have certain significance in the diagnosis of pancreatic cancer. Moreover, the diagnostic value of microRNAs is higher than that of traditional serum tumor markers [77]. Exosome is a kind of extracellular vesicles (EV), with a size of 50 ~ 150 nm. Exosome can be secreted under physiological or pathological state. Exosome contains DNA, microRNA, protein or other signaling molecules, and plays the role of exchanging information between cells. Since tumor cells can secrete exosomes 10 times more than normal cells, analysis of abnormal serum exosomes and their encapsulated molecules often has widely been application in the diagnosis for tumors [78]. With the ability to enter the peripheral circulatory system, circulatory tumor cells (CTC) vary in individual and express both epithelial and mesenchymal markers [79]. It has been reported that such cells can be detected in the peripheral blood of 40% ~ 100% of pancreatic cancer patients, which may be used for the early diagnosis of pancreatic cancer [80].

4.2 Imaging diagnosis

Multi-Detector Computed Tomography (MDCT) is now the most routinely performed for the diagnosis of suspicious pancreatic lesions, assessment of resectability and vascular invasion, and detecting metastatic lesions [81]. The appearances of pancreatic cancer in non-contrast CT scan include solid mass (84.2%), diffuse enlargement (13.3%) with a vague or uneven glandular appearance, usually of slightly lower or equal density. A pancreas-specific protocol with dual-phase or multi-phase dynamic contrast is usually used, including early arterial phase images, pancreatic phase images and portal venous phase images. Early arterial phase images are sensitivity in evaluating the tumor and peri-pancreatic arteries. Pancreatic phase images are sensitivity in evaluating pancreatic lesions, and portal venous phase images are sensitivity in evaluating the involvement of the portal vein, the superior mesenteric vein and other veins. Enhanced CT scan showed enhancement in the early stage, with a peak earlier than the liver, relative lack of blood, about 93% showed uneven low density, distal pancreatic atrophy and dilatation of the pancreatic duct [81].

In addition to showing the anatomical features of pancreatic tumors, MRI can also supply information about the metastatic lesions in lymph nodes and liver.

The weighted expression of T1WI was low or slightly low signal, while T2WI was slightly high or mixed signal for the tumor mass. The enhancement scan showed significant enhancement of the normal pancreas and only slight enhancement of the tumor. Diffusion-weighted imaging (DWI) is an MRI technique based on the Brownian motion of water molecules in tissue [82], which is greatly useful in differentiating mass-forming focal pancreatitis from pancreatic cancer [83, 84].

PET-CT can show the metabolic activity of the tumor, and has obvious advantages in the detection of pancreatic metastasis and the evaluation of systemic tumor load. Combination of PET-CT with endoscopic ultrasonography is useful for suspected pancreatic cancer diagnosis because of the high sensitivity of PET-CT and the high specificity of endoscopic ultrasonography (EUS) [85].

EUS is considered the most sensitive method for detecting early neoplastic in the pancreas, which is superior to MDCT [86]. A meta-analysis of 20 studies showed that the performance of EUS in PDAC diagnosis depended on the T stage. The sensitivity and specificity was 72% and 90% for T1–2 stage cancers and 90% and 72% for T3–4 stage cancer in EUS [87]. EUS can detect pancreas lesions as small as 2–3 mm [88]. In particular, EUS guided fine needle biopsy has become the most accurate method for the localization and qualitative diagnosis of pancreatic cancer.

4.3 Molecular imaging diagnosis

In 1999, Weissleder of Harvard University first proposed the concept of molecular imaging [89]. Molecular imaging is a biological process that can be observed, qualitatively and quantitatively analyzed in humans and other living organisms at the molecular or cellular level. It generally includes two-dimensional or three-dimensional images and quantitative maps of signals changing over time. The rise of molecular imaging has broken the limitation of traditional imaging in mainly reflecting the changes of anatomical structure, made modern medical imaging go deep into the microscopic level of living organisms, realized the extension of structural image to functional image, and provided an effective way for accurate medical disease diagnosis. Molecular imaging relies on advanced imaging equipment, highly sensitive and specific molecular imaging probes [89].

Several groups have investigated novel imaging agents that are coupled to 18F applied to PET own to its false positives (eg, benign causes of inflammation like pancreatitis) and false negatives (eg, non–18F fluorodeoxyglucose avid tumors). Other strategies to detect pancreatic cancer with molecular imaging agents include targeting proteins that are overexpressed by the cancer (eg, mesothelin), signaling pathways (eg, epidermal growth factor receptor), tumor stroma (eg, hedgehog signaling, vascular endothelial growth factor), and other targets that are associated with the disease (eg, plectin-1, MUC-1] [90]. Another molecular imaging method that is of interest for early detection is hyperpolarized MRI, which can identify metabolic aberrations in the pancreas that indicate precancerous lesions [91].

Researchers used PEG as shell, limiting Mn^{2+} calcium phosphate acid as nuclear build a lack of oxygen can be used in the tumor area imaging of molecular probes, after the probe enters the tumor, tumor area lower pH can cause lack of oxygen. The dissolution of calcium phosphate thus releases its limitations of Mn^{2+}, causing local T1 relaxation rate increase significantly, thus successfully mapping the hypoxia zone in the tumor to improve clinical tumor treatment effect [92]. In addition, the design of introducing the disulfide bond into the probe and being interrupted by increased glutathione (GSH) in vivo successfully realized the molecular level imaging (fluorescence, 19f-MRS, MRI, etc.) reflecting the redox state of the lesion area [93]. By introducing amino acid sequences that can be recognized and cut off by caspase-3, the probe can realize the aggregation of small molecule monomers under

the action of activated caspase-3, causing significant amplification of the imaging signal, and thus reflecting the occurrence of early apoptotic events in pancreatic carcinoma [94].

5. Conclusion

As one of the highest mortality cancer, pancreatic cancer is still a disaster disease. Novel diagnosis and therapy avenues should be developed to improve the survival rate of this disease.

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