Introduction
Chapter 1

Pancreatic cancer, an introductory overview, and outline of the thesis

Mina Maftouh, Godefridus J. Peters, Elisa Giovannetti

Department of Medical Oncology, VU University Medical Center, Amsterdam, the Netherlands

Manuscript in preparation in conjugation with Chapter 8
Chapter 1

Pancreatic cancer, an introductory overview

1.1. General introduction

An incidence rate nearly equal to its mortality rate demonstrates the aggressiveness and lethal nature of pancreatic cancer. Pancreatic ductal adenocarcinoma (PDAC) includes more than 90% of pancreatic cancers. This thesis focuses mainly on PDAC.

Most patients present with advanced disease (i.e., locally-advanced or metastatic) at diagnosis, and survival rate has not improved in the last decade, with less than 5% of patients alive five years after diagnosis. Gemcitabine is the most commonly used chemotherapeutic agent in the treatment of PDAC, however, this therapeutic regimen results in less than 12% response rate in the patients. Such dismal outcome can be explained by the lack of biomarkers for early screening/diagnosis, together with the aggressive biological behavior, characterized by early metastatic spread, and by the resistance to currently available chemotherapy regimens. Therefore, there is an urgent need to develop novel anticancer agents that either improves gemcitabine activity, within novel combinatorial regimens, or with a better efficacy than gemcitabine. Furthermore, in addition to the widespread genetic alterations, it is now apparent that epigenetic and metabolic mechanisms are also central to the evolution and progression of PDAC. Therefore, novel approaches targeting epigenetic and metabolic modifications are warranted to inhibit PDAC invasiveness and its chemoresistance.

Here we hypothesize that lack of knowledge about epigenetics and metabolic characteristics of PDAC, as well as the lack of drugs interacting synergistically with gemcitabine may have, at least in part, hampered clinical progress in the treatment of PDAC. Therefore, the research described in this thesis aimed to (1) investigate novel mechanisms underlying the aggressiveness and chemoresistance of PDAC, such as microRNA and metabolic alterations; (2) evaluate the efficacy of new anticancer agents and treatment strategies that can target these alterations, and could be readily translated to the clinic.

The aim of the current chapter is to give an introductory overview of this dismal disease. First, we summarize the clinical presentation and management of PDAC, continuing with a brief description of the anatomy of pancreas, and of the main genetics, epigenetics and metabolic characteristics of PDAC.

1.2. Pancreas anatomy

Pancreas is a gland organ found in all vertebrates. The pancreas is comprised of two separate functional units, exocrine and endocrine functions, which are involved in two major physiological processes: digestion and glucose metabolism, respectively (Figure 1). The exocrine pancreas consists of acinar and duct cells. The acinar cells produce digestive enzymes, which are secreted into the small intestine to convert food into smaller components (nutrients) that the body can assimilate. The ducts, which add mucous and bicarbonate to the enzyme mixture, form a network of increasing size, culminating in main and accessory pancreatic ducts that empty into the duodenum. The endocrine pancreas is made by four specialized cell types that are organized into compact islets embedded within acinar tissue, and secretes hormones into the blood stream. In particular, α- and β-cells
regulate the usage of glucose through the production of glucagon and insulin, respectively. δ-cells and PP cells produce somatostatin and pancreatic polypeptide, respectively, that modulate the secretory properties of the other pancreatic cell types [1].

1.3. Clinical presentation, diagnosis and management of PDAC

The symptoms of PDAC are non-specific, with abdominal pain, weight loss, general malaise, diarrhea, anorexia and vomiting as the most commonly reported complaints. Upon progression of the tumor, the pancreatic and bile ducts often become blocked, resulting in reduction of bile and pancreatic juice secretion into duodenum. Abdominal pain becomes more localized mainly in the upper middle of the abdomen, which is initially caused by tumor growth into the celiac and superior mesenteric plexus. Back pain is also reported, when the tumor progresses into the retroperitoneal plexus. Loss of body weight is most likely caused by the decreased food intake and fat uptake by small intestinal epithelium due to the decreased bile secretion. The energy consuming aerobic glycolysis of the tumor cells, known as Warburg effect, also contributes to the weight loss of the patients.

The most common sign of PDAC is painless jaundice (yellow tint to whites of eyes [sclera] or yellowish skin, possibly in combination with darkened urine), when a cancer of the head of the pancreas (75% of cases) obstructs the common bile duct as it runs through the pancreas. This may cause pale-colored stool and steatorrhea. Physical examination of
Chapter 1

PDAC patients often shows a palpable mass in the upper abdomen, lymphadenopathy, hepatomegaly and ascites. However, most of these signs usually indicate an advanced stage of tumor. Hematological abnormalities can present with non-specific mild anemia and hyperglycemia. Prothrombin time is often increased due to malabsorption of vitamin K, as a consequence of reduced fat absorption [2].

PDAC can lead to malfunctioning of the β-islets in the pancreas resulting in diabetes mellitus. Since at the time of diagnosis about 80% of PDAC patients have impaired insulin secretion [3], PDAC should be considered in differential diagnosis with respect to type II diabetes. Of note, a dysbalance in plasma glucose levels is often observed in early stages of PDAC, which can be considered a possible marker for diagnosis [4].

Carbohydrate antigen 19-9 (CA 19-9) is the most commonly used marker for therapeutic monitoring and detection of recurrent disease in PDAC [5]. However, CA 19-9 is significantly increased only in larger tumors, which makes it a disappointing tool for screening [6].

PDAC patients often shows a palpable mass in the upper abdomen, lymphadenopathy, hepatomegaly and ascites. However, most of these signs usually indicate an advanced stage of tumor. Hematological abnormalities can present with non-specific mild anemia and hyperglycemia. Prothrombin time is often increased due to malabsorption of vitamin K, as a consequence of reduced fat absorption [2].

PDAC can lead to malfunctioning of the β-islets in the pancreas resulting in diabetes mellitus. Since at the time of diagnosis about 80% of PDAC patients have impaired insulin secretion [3], PDAC should be considered in differential diagnosis with respect to type II diabetes. Of note, a dysbalance in plasma glucose levels is often observed in early stages of PDAC, which can be considered a possible marker for diagnosis [4].

Carbohydrate antigen 19-9 (CA 19-9) is the most commonly used marker for therapeutic monitoring and detection of recurrent disease in PDAC [5]. However, CA 19-9 is significantly increased only in larger tumors, which makes it a disappointing tool for screening [6].

Several imaging techniques are being used for diagnosis of PDAC, including ultrasonography (US) in combination with Doppler imaging, Computed Tomography (CT), and/or Positron Emission Tomography (PET) scan. In particular, US provides information on the presence or absence of a pancreatic tumor, bile duct enlargement and liver metastasis. However, it is difficult to accurately identify the stage of a tumor with US [7]. Although US is an appropriate initial imaging technique, thin-slice contrast-enhanced CT should always be used for diagnosis and staging. CT allows the visualization of the tumor in relation to surrounding structures. Finally, PET can also visualize PDAC. In the context of the high metabolic rate of tumor cells, this technique allows evaluation of metabolic activity of cells. Serrano and colleagues showed that PET scanning could be a useful tool for diagnosis and staging of PDAC, specifically in the cases where CT scanning fails to identify a discrete mass [8].

PDAC is staged according to the T(umor) N(ode) M(etastasis) classification of the American Joint Committee on Cancer. The TNM classification of PDAC is as follow: T1, tumor size ≤2.0 cm (limited to pancreas); T2, tumor size >2.0 cm (limited to pancreas); T3, tumor extends beyond the pancreas but does not involve the celiac axis or the superior mesenteric artery; T4, tumor has grown into these structures. N0 or N1 illustrate the absence or presence of lymph node metastases, respectively, while the tumor is designed as M0 or M1 when distant metastases are not detectable or detectable, respectively.

Tumors are usually divided into stages I, II, III or IV using the TNM classification. Stage I tumors have no lymph node/distant metastases, either T1 or T2 tumor grade. Stage II tumors either have T3N0M0 or T1/T2/T3 with lymph node involvement. Stage III tumors are T4 with either N0 or N1, while any tumor with M1 is stage IV. Currently, stage III/IV tumors are per definition unresectable [9].

1.4. Treatment

Treatment options for each patient with PDAC differ depending on tumor resectability. Currently, surgical resection remains the only potentially curative treatment for localized tumors that are confined to the pancreas. Patients undergo classical pancreaticoduodenectomy, distal pancreatectomy or total pancreatectomy, depending on the
tumor location. Most patients (≈80%) present with advanced disease (i.e., locally-advanced or metastatic) at diagnosis. The primary goals of treatment in this group of patients are survival prolongation and palliation [10]. Gemcitabine is being recommended for therapy as a treatment of choice since 1997, when it was shown to increase overall survival and improve quality of life [11,12]. Chemoradiotherapy has been used in USA since the Gastro-Intestinal Tumor Study Group trial demonstrated longer overall survival in the group treated with chemoradiation [13,14]. However a similar investigation failed in Europe. Chemoradiation therapy is not considered as a standard in treatment of PDAC patients in western countries [15,16], but recent studies have been initiated by the Dutch Pancreatic Cancer Group to evaluate radiochemotherapy versus immediate surgery for resectable and borderline resectable PDAC [17]. Over the last few decades very few of the doublets in combination with gemcitabine have succeeded. Erlotinib-gemcitabine was the only combination that got approved, but on average, this treatment provided only one month of additional life. However, a recent landmark phase III trial demonstrated that the combination of oxaliplatin, irinotecan, fluorouracil, and leucovorin (FOLFIRINOX) is an option for the treatment of metastatic patients with good performance status [18]. The objective response rate was 31.6% in the FOLFIRINOX group versus 9.4% in the gemcitabine group (P<0.001). Moreover, the median overall survival in the FOLFIRINOX group was 11.1 months as compared with 6.8 months in the gemcitabine group (hazard ratio for death, 0.57; 95% confidence interval [CI], 0.45 to 0.73; P<0.001). Similarly, in a recent randomized phase III trial, the regimen of nab-paclitaxel (Abraxane) plus gemcitabine showed better antitumor activity with respect to gemcitabine alone [19]. However, these therapeutic strategies are associated with increased toxicity and further studies are needed to evaluate novel strategies to improve gemcitabine efficacy against PDAC.

1.5. Tumor biology
1.5.1. Pathogenesis of PDAC
PDAC develops in a step-wise manner in which non-malignant pre-invasion lesions progress to an invasively growing tumor (Figure 2). Shortly, pancreatic intraepithelial neoplasia (PanIN) lesions are the pre-invasive form of tumor with microscopic dimensions, which is found in smaller pancreatic ducts. These lesions are classified into different stages, PanIN-1, PanIN-2 and PanIN-3, on the basis of cellular and nuclear atypia and architectural changes. PanIN-1 lesions show little cytonuclear atypia and have retained their cellular polarity. This type of lesion is subdivided into two groups: PanIN-1A and PanIN-1B based on whether the cells have a squamous-like fashion (1A) or a micropapillary architecture (1B). PanIN-2 lesions show evident cytonuclear atypia and infrequent mitoses, while PanIN-3 lesions, also referred to as carcinoma in situ, show all the hallmarks of cancer except the invasive growth [20,21]. Other recognized precursor lesions of adenocarcinoma (intraductal papillary mucinous neoplasm [IPMN] and mucinous cystic neoplasm [MCN]) likely harbor a distinct compendium of genetic alterations in their path to invasive cancer. PDAC is also characterized by the presence of a dense stroma of fibroblasts and inflammatory cells, termed desmoplasia. This tumor-associated desmoplasia has been described in recent years as being complicit in PDAC growth. In normal tissues, the stroma provides nutrients and regulatory signals for proper cellular polarity and function. However,
following oncogenic transformation, the stromal compartment is conscripted to provide stimulatory signals and protection to tumor cells. Several types of tumor-stroma interactions have been implicated as having the potential to promote PDAC invasion and metastasis. Cancer cell derived growth factors, such as fibroblast growth factors (FGFs), transforming growth factor-beta (TGF-β), insulin-like growth factor-1 (IGF-1) and platelet-derived growth factor BB (PDGF-BB), become sequestered within the stroma, which thus acts as a storage site for these factors. The invading cancer cells produce matrix metalloproteinases (MMPs) that release these growth factors [22].

1.5.2. Genetic alterations

The progression from histologically normal epithelium to invasive carcinoma is associated with the accumulation of genetic alterations. Telomere shortening is one of the first events in the progression to a pre-invasive lesion and eventually to PDAC, which results in chromosomal instability and allows accumulation of genetic alterations [23]. Telomeres are repeated sequences at the end of linear chromosomes, which prevent fusion between chromosomes. Short telomeres can result in ring chromosomes and dicentric chromosomes that form a so-called anaphase bridge during mitosis. Breakage of anaphase bridges generates highly recombinogenic free DNA ends, leading to chromosomal rearrangements. This event, which is called “anaphase bridge-breakage-fusion cycle”, is repeated, thereby leading to genetic instability that forms the basis of tumor development.

Several mutations have been observed either in oncogenes, e.g., KRAS, or tumor suppressors genes, e.g., CDKN2, TP53, DPC4/Smad4 and BRCA2. KRAS, CDKN2, TP53, and DPC4 are commonly referred as PDAC “driver genes”. In particular, KRAS is one of the main players in the development of PDAC. This gene is mutated in the majority (90%) of PDACs. KRAS encodes a member of the RAS family of guanosine triphosphate (GTP)-binding proteins that mediates a range of cellular functions, including proliferation, cell survival, cytoskeletal remodeling, and motility. In addition to its role in PDAC initiation, constitutive RAS signaling appears to be important also for PDAC maintenance.

The most commonly affected tumor suppressor gene in PDAC is CDKN2A, which encodes p16 protein. Inactivation of CDKN2A is observed in more than 95% of patients [24]. TP53 is another important tumor suppressor gene, which is inactivated in 25-60% of cases [25]. The gene encodes p53, a protein involved in cell cycle arrest and apoptosis upon cytotoxic stress. This gene is mainly mutated in PanIN-3 in transition to invasive growth, as a late event in PDAC development [26]. Moreover, the activity of p21 is lost in 40-60% of PDACs. The p21 is activated after the stimulation of p53 that inhibits the activity of cell proliferation-stimulating complex containing the protein cyclin D [27,28]. DPC4 mutations are observed in approximately 55% of patients. These mutations occur in late stages of tumor development, mainly in Pan IN-3 [29]. DPC4 encodes Smad4 that is part of the transforming growth factor (TGF)-beta signaling. TGF-beta is one of the most important signaling pathways involved in cell cycle inhibition, differentiation, apoptosis, and angiogenesis [30]. Several studies have supported both the diagnostic and prognostic values of Smad4 expression in PDAC [31,32].

Recent advances in genome sequencing have revealed a complex picture of genetic interaction in the initiation and progression of PDAC [33]. This study showed frequent genetic alterations in a wide variety of core signaling pathways (e.g., Wnt and TGF-beta),
and re-activation of embryonic signaling pathways (e.g., Notch and Hedgehog). In particular, recent studies have demonstrated that the activation of Wnt/β-catenin pathway is required for initiation and progression of PDAC [34]. Thus, inhibition of Wnt/β-catenin pathway by novel anticancer agents might have a potential therapeutic impact on suppression of tumor progression.

Importantly, it has been shown that galectin-4 (Gal-4) regulates Wnt/β-catenin pathway in several tumor types, including colorectal cancer [35]. Galectins (β-galactosyl binding lectins) are a large family of proteins characterized by carbohydrate recognition domain with a broad variety of functions, including cell-cell/matrix adhesion and growth regulations [36-37]. Galectins are aberrantly expressed in several human cancer and associated with cancer initiation, progression [38-39] or cancer-associated stromal cells [40]. In cancer progression, galectins are involved in differentiation, adhesion, migration, angiogenesis, malignant transformation, apoptosis and cancer drug resistance [38-41-42-43]. Satelli and colleagues showed that Gal-4 is down-regulated in colorectal cancer [35], while the enforced expression of Gal-4 in colorectal cancer cells inhibited Wnt/β-catenin pathway, suggesting its role as a tumor suppressor gene. Thus, studies investigating the biological role of Gal-4 on invasive behavior of several representative primary PDAC cell culture as well as its impact in regulation of Wnt/β-catenin pathway in pancreatic cancer proliferation are warranted, as reported in the chapter 5.

The phosphatidylinositol-3 kinase (PI3K)/Akt pathway is another core signaling pathway in PDAC [33]. In particular, the serine/threonine kinase Akt, which is coded in three highly homologous isoforms (Akt1, Akt2 and Akt3), is overexpressed in more than 40% of PDAC [44-45]. Moreover, activation of Akt is a frequent has been correlated to poor prognosis [46-47], while inhibition of the PI3K/Akt pathway sensitizes pancreatic cancer cells by increasing apoptosis both in vitro and in vivo [48]. However, studies on the activity and the molecular mechanisms triggered by the novel Akt inhibitor perifosine in PDAC cells are still missing. Therefore, in the chapter 6 we investigated the expression of phospho-Akt in PDAC tissues and cells, and characterized several factors underlying the synergistic interaction of perifosine with gemcitabine.

1.5.2.1. Epigenetics

Although defined core-signaling pathways are altered in the majority of PDAC, the role of the many identified mutations and genetic aberrations remains unclear. Further understating of these changes at functional level will be critical for the identification of new targets for both early diagnosis and more effective treatment of PDAC. Moreover, beside in addition to classical genetics, regulation of gene expression is also modified by 'epigenetic' alterations including chromatin remodeling and histone variants, DNA methylation, the regulation of polycomb group proteins, and the epigenetic function of non-coding RNA, including microRNA (miRNA).

Therefore, in the chapters 2, 3 and 4 we studied the biological and clinical implications of key miRNAs and of the histone-lysine N-methyltransferase EZH2 in PDAC.
Figure 2. Pancreatic precursor lesions and genetic and epigenetics events involved in pancreatic adenocarcinoma progression. The model illustrates normal duct epithelium progressing to infiltrating cancer (left to right) through a series of histologically defined precursors (PanINs). The various genetic events are listed and divided into those that predominantly occur early or late in PDAC progression. “+” and “−” indicate presence and absent of miRNA expression in normal ducts, respectively. “Δ” and “△” indicate changes in level of expression (2-fold to 10-fold and 0.1-fold to 0.5-fold, respectively). “□□” and “□□□” indicate changes in the level of expression (>10-fold and <0.1-fold, respectively).

During pancreatic cancer development a variety of signaling pathways participate in multiple stages of pancreatic tumorigenesis from early precursor lesions. According to significantly involved signaling pathways tumor cell survival, angiogenesis, invasion, desmoplasia, and tumor immune response are affected, respectively. Ensuing alterations together with epigenetic changes are strongly involved in promoting tumor progression and chemotherapy resistance, and thus provide potential therapeutic targets in pancreatic cancer.
1.5.2.1.1. microRNA

Several studies have evaluated the complex genetic networks and transcriptomics alterations underlying the development and progression of PDAC [49-50]. The recent discovery of miRNAs has provided additional insights potentially explaining the gap that exists between tumor genotype and phenotype.

MiRNAs are small (19-23 nucleotides, single strand), non-protein-coding, endogenous RNAs playing a pivotal role in the regulation of gene expression at the post-transcriptional level [46]. The primary miRNA (pri-miRNA) is transcribed by RNA polymerase II, cleaved by Drosha RNase III endonuclease into the miRNA precursor (pre-miRNA), then transported from the nucleus to the cytoplasm by the Exportin-5 protein and further cleaved by Dicer into the mature miRNA [51]. These small RNAs, together with Argonaute (Ago) proteins, form the RNA-induced silencing complex (RISC) and bind to their target mRNA through partial complementarity, leading to inhibition of mRNA translation [52-55].

MiRNAs have been shown to regulate many biological processes, but they are also involved in the pathogenesis of several human diseases, including PDAC, as shown in Figure 2. Moreover, since miRNAs are stable and detectable in human blood they could be useful as diagnostic or prognostic markers. Understanding the role exerted by specific miRNAs in the initiation and progression of PDAC is increasing, but further studies on miRNAs affecting gemcitabine activity are warranted. In a high-throughput miRNA array, miRNA-211 emerged as the best discriminating miRNA, with high expression associated with long survival. Therefore, in the chapter 4 we further explored the biological role of miRNA-211 might affect gemcitabine chemosensitivity, using PDAC cells with different migratory abilities.

1.5.2.1.2. EZH2

Enhancer of Zeste Homolog 2 (EZH2) is a pivotal catalytic subunit of the Polycomb group (PcG). PcG proteins can repress gene expression by forming multiple complexes leading to histones methylation, thus resulting in epigenetic control of gene expression [56]. In particular, EZH2 can silence several tumor suppressor genes by trimethylation at lysine 27 of histone H3, playing a key role in tumor development [57]. Furthermore, EZH2 is crucial for cancer stem cells self-renewal in several cancer types [58], including PDAC, where EZH2 overexpression has been associated with decreased E-cadherin expression, invasion and poor prognosis [59-60]. EZH2 is also an important factor in PDAC cell chemoresistance, since EZH2 depletion by RNA interference or inhibition by DZNep sensitized PDAC cells to gemcitabine [59, 61].

Recently EZH2 expression emerged as a prognostic factor for in radically resected PDAC patients [22]. However, since (1) blood samples are much easier to obtain, especially for advanced cancers, and (2) recent studies suggested a role for candidate polymorphisms of EZH2 in colorectal cancer prognosis [62], in chapter 2 we performed a pharmacogenetic study aimed at exploring the prognostic value of EZH2 expression and two candidate EZH2 polymorphisms in locally-advanced or metastatic PDACs.
1.5.3. Metabolic reprogramming

Pancreatic cancer is also characterized by metabolic-switch to anaerobic-glycolysis, which has the main goal to provide sufficient energy for cell proliferation [63]. This reprogramming in metabolic pathways happens due to different reasons including mutations in oncogenes and/or tumor suppressor genes, as well as inadequate vascularization which prepares a hypoxic and low nutrient microenvironment in and around PDAC cells [64].

Several members of the glucose metabolism pathway are overexpressed in human pancreatic cancers. Besides Glut1 and aldolase A, also glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is significantly up-regulated in PDAC as well as enolase and hexokinase II. Glycolysis results in the production of pyruvate, which normally is further metabolized via the citrate cycle in aerobic metabolism. However, under hypoxic conditions, pyruvate is converted to lactate by lactate dehydrogenase A (LDH-A), directing glucose carbon away from the tricarboxylic acid cycle and fatty-acid synthesis [65]. LDH-A is associated to the viability of tumor cells in hypoxia and overexpressed in metastatic cancer cells [66,67]. Tumor cells secrete lactate since intracellular accumulation of the molecule would inhibit LDH-A enzyme activity. Secreted lactate lowers the pH of the extracellular environment, and might directly activate HIF-1α even in normoxic conditions. Additionally, lactate might be transported to the liver, where one new glucose molecule can be generated from two lactate molecules. This process is called the Cori cycle. It is an inefficient pathway since the production of one glucose molecule needs the energy from six ATP molecules while only two ATP molecules are generated during anaerobic glycolysis. This futile cycle might also have a significant contribution to PDAC cachexia.

Several studies have shown the prognostic relevance of LDH in different tumor types, including PDAC [68]. Furthermore, reduction of LDH-A by siRNA or its inhibition by small-molecule FX11 inhibited the progression of sizable human lymphoma and pancreatic cancer xenografts [69]. The inhibition of LDH-A can be achieved without influencing the energetic balance of normal tissues [70], and has not been shown to raise major side effects, in agreement with the observation that hereditary LDH-A deficiency does not provoke any symptoms under ordinary circumstances in humans [71].

Therefore the glucose metabolism pathway presents several possibilities for therapeutic intervention, and in the chapter 7, we evaluated the activity of two of the most promising LDH-A inhibitors [66, 72], in combination with gemcitabine in pancreatic cancer cells.

In addition, increased Akt pathway signaling has been shown to be directly correlated with increased rates of glucose metabolism observed in cancer cells versus normal cells [73]. These observations have led to the proposal that inhibition of Akt signaling would inhibit glycolysis and increase hydroperoxide production, which would preferentially kill tumor cells versus normal cells via oxidative stress. Therefore our studies on the novel Akt inhibitor perifosine (chapter 6) might have also a significant value for the modulation of the Warburg effect and to unravel how this phenomenon may be exploited to enhance chemosensitivity in PDAC therapy.
1.5.4. Conclusions and future prospects

Extensive research in the last two decades has resulted in a solid understanding of pancreas carcinogenesis. Unfortunately these findings have not led to major advances in diagnostics or treatment, and PDAC is still the most lethal among solid tumors.

However, it has become also apparent that PDAC is an epigenetic disease, as it is a genetic disease, and novel epigenetic biomarkers, such as miR-211, may help stratify PDAC patients. In addition, epigenetic modifications are reversible through specific drugs, such as EZH2 inhibitors. Preclinical studies suggest that these drugs may reverse chemoresistance, and an epigenetic approach may pave the way to more effective therapeutic strategies.

Similarly, understanding the altered metabolism and main pathways in PDAC, with particular emphasis on blocking the pancreatic fuel supply or pro-invasive signaling with small-molecule inhibitors of LDH, Akt or Wnt could lead to better strategies to fight this disease.

The emphasis of future research on understanding of epigenetics mechanisms and metabolic changes leading the aggressive behavior of PDAC, should prompt the development of new (targeted) treatments that increase survival rates according to the characteristics of each patient (i.e., personalized medicine).
1.6. Outline of the thesis

The research described in the current thesis was mainly focused on: (1) the elucidation of molecular mechanisms underlying the aggressiveness and chemoresistance of PDAC, in order to identify prognostic and predictive markers of treatment response; and (2) the study of the therapeutic potential of novel anticancer agents to test new (targeted) treatment strategies.

In chapter 2, we evaluate the prognostic value of the key epigenetic factor EZH2, by analysis of its expression and of candidate polymorphisms in locally-advanced or metastatic PDAC patients.

In chapter 3, we describe the implication of miR-211 activity in human diseases and neoplasms, including PDAC.

In chapter 4, we evaluate the role of miR-211 in the modulation of gemcitabine cytotoxicity and ribonucleotide reductase in PDAC cells.

In chapter 5, we evaluate the role of Galectin-4 and Wnt/β-catenin signaling pathway in the invasive behavior of PDAC cells and xenografts, as well as the chemosensitivity of PDAC cells to novel Wnt inhibitors.

In chapter 6, we explore the prognostic value of phospho-Akt in PDAC resected patients and evaluate the therapeutic potential of the novel Akt inhibitor perifosine in combination with gemcitabine in PDAC cells.

In chapter 7, we investigate the antiproliferative activity of novel lactate dehydrogenase inhibitors combined with gemcitabine against PDAC cells in hypoxia.

In chapter 8, we discuss the results presented in this thesis. Moreover, we provide our view on the future perspectives in PDAC research.

Finally, chapter 9 contains a summary in English, Dutch and Persian.
1.7 References

37. Ma J, Sawai H, Matsuo Y, et al. IGF-1 mediates PTEN suppression and enhances cell invasion and...


