**Summary**

Pancreatic cancer is an international problem because of its increasing incidence worldwide. The incidence and age-adjusted mortality rates are almost equal, underscoring the aggressive nature of the disease. Although efforts are being made to unveil the principles governing the initiation and progression of this cancer, and to identify the factors that confer its particular aggressiveness, the exact succession of molecular events underlying the development of this devastating malignancy has remained unsolved. The management of pancreatic cancer is, therefore, an ongoing challenge. Many translational studies were presented at the annual meeting of the American Society of Clinical Oncology (ASCO) this year. The author summarizes few of them including: polymorphisms of genes involved in gemcitabine metabolism correlate with prognosis in patients receiving neoadjuvant therapy for pancreatic cancer, diagnostic performance of MUC1 for pancreatic ductal adenocarcinoma, and use of whole genome expression analysis of pancreatic adenocarcinoma to predict prognosis after surgery. Pancreatic cancer remains a dismal disease and early diagnostic markers and therapeutic targets are urgently needed.

**Introduction**

Adenocarcinomas of the ductal phenotype constitute over 90% of pancreatic cancer, and other tumours of ductal type, such as mucinous cystic tumours, serous cystic tumours and IPMTs make up another 5-7%. A number of factors, including hereditary, environmental, occupation, and social factors are now recognized as potential contributors to the development of pancreatic cancer. It is a daunting challenge for modern medicine as the survival rates for patients diagnosed with metastatic pancreatic cancer range from 4 to 6 months on average. The lethality of the disease is best illustrated by its poor 5-year survival rate of a mere 5% [1]. The highest cure rate only occurs if the tumour is truly localized to the pancreas; however, this stage of disease accounts for fewer than 20% of all cases. The inability to diagnose pancreatic cancer at an early, localized, and curable stage has contributed to poor prognosis. The silent course of pancreatic cancer and its explosive fatal outcome have hindered studies of identification of early biochemical and genetic alterations that could help us diagnose the disease at a curable stage and develop successful therapeutic strategies. Thus, pancreatic cancer remains a dismal disease and early diagnostic markers and therapeutic targets are urgently needed.

**Polymorphisms of Genes Involved in Gemcitabine Metabolism Correlate with Prognosis in Patients Receiving Neoadjuvant Therapy for Pancreatic Cancer**

**Pharmacology of Gemcitabine**

Gemcitabine (2',2’-difluorodeoxycytidine) is a fluorine-substituted deoxycytidine analog. It
requires intracellular activation by deoxycytidylate kinase to the monophosphate form with eventual metabolism to the cytotoxic nucleotide metabolite difluorodeoxycytidine 5'-triphosphate (dFdCTP). Antitumor activity of gemcitabine is determined by a balance between intracellular activation and degradation and the formation of cytotoxic triphosphate metabolites. Incorporation of dFdCTP metabolite inhibits several DNA polymerases which in turn, interferes with DNA chain elongation, DNA synthesis, and DNA repair. Difluorodeoxycytidine diphosphate (dFdCDP) metabolites inhibit the enzyme ribonucleotide reductase, resulting in decreased levels of essential deoxyribonucleotides for DNA synthesis and function [2]. Incorporation into RNA resulting in alterations in RNA processing and mRNA translation (Figure 1).

Equilibrative nucleoside transporters (hENT), and concentrative nucleoside transporters (hCNT) are proteins that transport gemcitabine to intracellular targets. There are over 58 hCNT single nucleotide polymorphisms (SNPs) identified with three SNPs with probable functional impact. In vitro studies revealed that gemcitabine is mostly transported to intracellular targets via hENT1 transporters [3]. At least 24 hENT SNPs are also being identified however, the functional impact of these SNPs are being investigated [4, 5]. There are in vitro data showing that gemcitabine sensitivity may be significantly altered by inhibiting these transporter proteins [6, 7]. There are studies also suggesting that high expression of hENT1 is associated with survival benefits in pancreatic cancer patients [8].

Deoxycytidylate kinase (dCK) is viewed as a rate-limiting enzyme in activating gemcitabine after cellular uptake by transporters. Decreased expression of this enzyme has been reported to be one of the mechanisms of gemcitabine resistance. Correlation between pancreatic cancer's sensitivity to gemcitabine and dCK has been well described [8, 9, 10]. dCK enzyme activity modulating agents are being investigated to design a combination regimen that will optimize gemcitabine's antitumor activity [11, 12].

Gemcitabine is inactivated by cytidine deaminase (CDA) and high expression of this enzyme has been associated with gemcitabine resistance and shorter survival [12, 13]. CDA is a polymorphic enzyme and there is one variant allele (208G>A (3*, A70T)) that was identified in Japanese population (allele frequency of 0.037) that had functional impact of CDA. 3* allele appeared to change pharmacokinetic parameters and plasma CDA activities significantly leading to decreased clearance of gemcitabine and increased toxicity. More polymorphisms are being identified and their functional impact will need to be examined [4].

**Study**

Javle et al. investigated genetic variations in gemcitabine transport and metabolism that may affect the clinical response, toxicity, and prognosis of pancreatic cancer patients treated with gemcitabine [14]. The investigators evaluated 17 SNPs of 8 genes (CDA, dCK, ribonucleotide reductase M1 (RRM1), deoxycytidylate deaminase (DCTD), hCNT1, hCNT2, hCNT3, and hENT1) involved in gemcitabine metabolism in a homogeneous population of 126 patients with resectable pancreatic cancer treated with neoadjuvant gemcitabine-based chemotherapy plus radiation therapy.

![Gemcitabine Metabolism](Figure 1. Gemcitabine metabolism. Adapted from Saif MW [18]).
They found that:

- six of the 17 SNPs had a borderline-significant effect on overall survival (P<0.1, log-rank test);
- a significant combined genotype effect on overall survival was observed for the metabolic or target gene SNPs CDA 111CC, CDA -76AA, RRM1 42GG, and DCTD -47CT (P<0.001) and for the transporter gene SNPs hCNT1 -16AA/AG, hCNT2 -17CC, and hENT1 913CC (P=0.006);
- CDA C111T and dCK C-1205T genotypes were also significantly associated with toxicity (P=0.05 and P=0.03, respectively);
- CDA 111CC and dCK -1205TT alleles that were associated with poorer survival actually conferred less toxicity.

These findings suggest that polymorphic variants of gemcitabine metabolic genes play an important role in affecting the efficacy of gemcitabine treatment for patients with pancreatic cancer. Progress into a better management of patients with pancreatic cancer will be obtained only with large well-designed prospective clinical trials in which a direct comparison is done between patient treatment based on conventional (empiric) criteria and treatment selection suggested by analysis of the genetic background of tumors.

**Diagnostic Performance of MUC1 for Pancreatic Ductal Adenocarcinoma: A Meta-Analysis**

The early diagnosis of pancreatic cancer, as well as distinguishing between chronic pancreatitis and malignant pancreatic disease, remains still a clinical problem. Presently, there is no specific tumor marker for diagnosing pancreatic cancer. Mucin-associated marker like CA 19-9 are the most widely available pancreatic cancer tumor marker, but its value as a screening marker is limited by its reduced specificity.

**Mucins**

Mucins (MUCs) are heavily glycosylated, high molecular weight glycoproteins with an aberrant expression profile in various malignancies [15]. As main contributors to the rheologic properties of the mucus, mucins were thought to have the sole functions of protecting and lubricating epithelial surfaces. Following the development of molecular biological methods, however, a wide range of mucin structures became available and gave rise to a plethora of biochemical definitions of mucins. As the diversity of mucin structures grew in importance, a variety of functions were assigned accordingly. At present, a total of 21 genes have received the appellation MUC: MUC1-2, MUC3A, MUC3B, MUC4, MUC5AC, MUC5B, MUC6-13, MUC15-20 [15, 16]. The 14 classical mucins can be further grouped into two subfamilies:

- secreted; and
- membrane-bound.

Typically, secreted mucins are expressed exclusively by specialized epithelial cells, are secreted in the mucus, and display a restricted expression pattern within the human body. Among these, MUC2, MUC5AC, MUC5B, and MUC6 are expressed in the pancreas either under normal physiologic or tumoral conditions. The membrane-bound mucins are composed of MUC1, MUC3A, MUC3B, MUC4, MUC11, MUC12, MUC16, and MUC17. The membrane-bound mucins they can be expressed in four distinct forms:

1. membrane-anchored;
2. soluble (proteolytic cleavage of the membrane-bound form);
3. secreted (alternatively spliced variants); and
4. lacking the tandem repeat array (alternatively spliced variants).

MUC1 may inhibit cell-cell and cell-stroma interactions and function as a signal transducer, participating in cancer progression. In contrast, MUC2 is normally found only in goblet cells, where it contributes to the protective barrier function of these cells. Among the membrane-bound mucins, MUC1 and MUC4 are the two main mucins associated with pancreatic cancer. Previous
studies found that MUC1 might be a valuable tumor marker for early diagnosis of pancreatic cancer, but there was no convincing evidence [15, 16]. Jiang et al. performed a meta-analysis the role of MUC1 in diagnosing pancreatic ductal adenocarcinoma [17]. All studies on early diagnosis of pancreatic cancer with MUC1 were comprehensively searched in MEDLINE, EMBASE, CBMdisc and CNKI database using the keywords: pancreatic cancer, pancreatic neoplasms, pancreatic carcinoma, pancreatic adenocarcinoma, pancreas, MUC1, mucin-1. A quantitative meta-analysis was carried out. A total of 1,363 patients from 18 studies were included in the meta-analysis. Pooled accuracy indicators were:

- sensitivity: 0.83 (95% CI: 0.81-0.86);
- specificity: 0.63 (95% CI: 0.59-0.66), and
- diagnostic odds ratio (DOR): 20.44 (95% CI: 9.53-43.85)
- AUC of summary receiver operating characteristic (SROC) curve: 0.8879
- Q* index of SROC: 0.8185

The meta-analysis suggested that the mucins expression profile in pancreatic ductal adenocarcinoma has value for the diagnosis of pancreatic cancer.

**Use of Whole Genome Expression Analysis of Pancreatic Adenocarcinoma to Predict Prognosis after Surgery**

Pancreatic adenocarcinoma remains an important cause of malignancy-related death and is the eighth most common cancer with the lowest overall 5-year relative survival rate. Surgery is the only potential cure for patients with resectable pancreatic adenocarcinoma. However, only 20% of patients are respectable and even after the surgical resection, most patients succumb to recurrence. There, identifying new molecular markers and candidates for new therapeutic regimens is critical. Collisson et al. compared gene expression profiles associated with long and short-survival derived from microdissected formalin-fixed paraffin-embedded (FFPE) pancreatic cancer specimens to assess for prognostic information in resected tissues [13]. Among 29 specimens, 19 were from long (more than 300 days) and 10 from short (less than 300 days) survivors. Samples from pancreaticoduodenectomy for non-neoplastic diagnoses (n=8) were handled similarly. Differentially expressed gene lists were determined as described [13]. Between the groups, adenocarcinoma samples were clearly distinguishable from normal specimens using unsupervised analysis. The investigators identified about 300 differentially expressed genes between the long and the short survivor groups (P=0.005). Adaptive linear splines identified multiple genes, the expression levels of which were strongly correlated with survival duration (top gene P<0.001; false discovery rate: 0.03%). The leave-one-out cross-validation (LOOCV) analysis confirmed both methods.

This study aimed at gene profiling revealed that microdissected pancreatic ductal adenocarcinoma FFPE tissue is feasible and yields data indicative of prognosis after surgery. The change in genes could explain the disparity in outcomes seen after intended curative resection for pancreatic cancer is largely tumor-intrinsic. These results provide new insight into the study of human pancreatic cancer, including metastasis, and ultimately may lead to improving early diagnosis and discovering innovative therapeutic approaches for cancer.

**Keywords** gemcitabine; Gene Expression; Genome; Mucins; Pancreatic Neoplasms; Pharmacogenetics; Therapeutics

**Abbreviations** CDA: cytidine deaminase; DCTD: deoxyctydylate deaminase; dCK: deoxycytidine kinase; dFdCDP: difluorodeoxycytidine diphosphate; dFdCTP: difluorodeoxycytidine 5'-triphosphate; FFPE: formalin-fixed paraffin-embedded; hCNT: human concentrative nucleoside transporters; hENT: human equilibrative nucleoside transporters; SNPs single nucleotide
polymorphisms; RR: ribonucleotide reductase; RRM1 and RRM2: ribonucleotide reductase M1 and M2

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