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Molecular alterations contributing to pancreatic cancer chemoresistance

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ABSTRACT

Pancreatic ductal adenocarcinoma (PDAC) is one of the most common causes of cancer-related death all over the world. This disease is difficult to treat and patients have an overall 5-year survival rate of less than 5%. Although two drugs, gemcitabine (GEM) and 5-fluorouracil (5-FU) have been shown to improve the survival rate of patients systematically, they do not increase general survival to a clinically acceptable degree. Lack of ideal clinical response of pancreatic cancer patients to chemotherapy is likely to be due to intrinsic and acquired chemoresistance of tumor cells. Various mechanisms of drug resistance have been investigated in pancreatic cancer, including genetic and epigenetic changes in particular genes or signaling pathways. In addition, evidence suggests that microRNAs (miRNAs) play significant roles as key regulators of gene expression in many cellular processes, including drug resistance. Understanding underlying genes and mechanisms of drug resistance in pancreatic cancer is critical to develop new effective treatments for this deadly disease. This review illustrates the genes and miRNAs involved in resistance to gemcitabine in pancreatic cancer.

1. Introduction

Pancreatic adenocarcinoma is a most common cause of cancer related mortality all over the world. Suggested reasons for low survival rate among the pancreatic cancer patients are late diagnosis due to lack of reliable early detection markers, highly invasive and metastatic nature, lack of effective therapies, and acquisition of resistance during therapy. Treatment options for pancreatic cancer comprise of surgery, radiation and chemotherapy. Although initial treatment is successful for the majority of patients, most responders eventually become resistant to a wide range of chemotherapeutic agents.

Gemcitabine (GEM) and 5-fluorouracil (5-FU) have been proved effective against pancreatic cancer. 5-FU is the first approved chemotherapeutic drug and is widely used as an adjuvant and neoadjuvant chemotherapeutic agent to treat pancreatic cancers [1]. Since then, GEM has been used as the first line chemotherapeutic drug for this perilous cancer. Chemotherapy does not always provide any survival benefits for pancreatic cancer though it still remains the standard treatment for affected patients [2].

Moreover, combination of gemcitabine with other chemotherapeutic agents like 5-FU, cisplatin, oxaliplatin, capecitabine as well as biological agents like erlotinib, cetuximab, and bevacizumab has been reported [1].

Pharmacogenomics aims to determine which specific drug or combination of drugs would be effective and safe for a person based on their genome sequence or gene expression profile. However, most pharmacogenomic researches have focused on the role of single nucleotide polymorphisms (SNPs), and miRNA pharmacogenomics has yet to make a synergistic impact on molecular medicine [3]. Epigenetic modifications affect the expression of genes that play an important role in drug responsiveness. These changes include methylation of CpG-rich islands in the genome, histone modifications and changes in miRNA expression [4].

The influence of miRNAs on the regulation of drug-related genes is an important aspect of pharmacogenomics that could affect the chemotherapy regimen. MiRNAs are endogenous, single-stranded, non-coding RNA molecules composed of 18–25 nucleotides modulating gene expression. Currently, more than 1500 mature human miRNAs have been identified in mirBase, a database

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containing published miRNA sequences and annotation (miRBase 21, (miRBase 21, http://www.mirbase.org/). MiRNAs recognize their targets by binding to 3′UTR of mRNAs completely or partially, which inhibits translation and/or facilitates the degradation of target mRNA. MiRNAs are predicted to control the activity of approximately half of all protein-coding genes in cellular processes of mammals, including differentiation, development, proliferation, and apoptosis, which could promote and/or inhibit progression of human diseases like cancer [5,6]. In clinical cases of pancreatic cancer, miRNA profile can also influence drug responsiveness [7]. Additional cohort studies or in vitro assays on pancreatic cancer cells may provide an opportunity to find new candidate miRNAs for successful treatment in this deadly disease.

Until now, different mechanisms have been proposed for drug resistance in pancreatic cancer, like tumor microenvironment, alterations in individual genes or signaling pathways, and presence of highly resistant stem cells. The genes play a role in nucleoside transport, metabolism, cell cycle regulation, proliferation or apoptosis.

In order to design new therapeutic strategies and overcome drug resistance in pancreatic cancer, identification of genetic and epigenetic mechanisms involved in drug resistance seems to be critical. This review summarizes the genes, microRNAs and different mechanisms responsible for resistance to gemcitabine in pancreatic cancer and could be helpful in overcoming drug resistance.

1.1. Gemcitabine as first line chemotherapy for pancreatic cancer

Originally, gemcitabine was investigated as an antiviral agent [8]. Later, on the basis of its impressive in vitro and in vivo antitumor activity, FDA approved it as the first-line chemotherapy drug for patients with locally advanced or metastatic pancreatic adenocarcinoma in 1997 [9]. It is a deoxycytidine analog with several modes of action inside the cells. Cellular uptake of gemcitabine is mediated by a family of integral membrane proteins termed human nucleoside transporters (hNTs), which overcome the inherent barrier to diffusion imposed by hydrophilic nature of this nucleoside analog. Two types of hNTs are recognized, which are different according to mechanisms of transport, namely equilibrative sodium independent and concentrative sodium dependent (hENT and hCNT, respectively). It has been demonstrated that the majority of gemcitabine uptake is mediated by hENT1 and, to a lesser extent, by hENT2, hCNT1 and hCNT3 [10].

1.2. Metabolism and deactivation of GEM

As a prodrug, 2′,2′-difluoro 2′-deoxycytidine (dFdC) must be metabolized to the active triphosphate form of gemcitabine (2′, 2′-difluoro-2′-deoxycytidine triphosphate; dFdCTP). Inside cytoplasm, gemcitabine is phosphorylated by deoxy cytidine kinase (dCK) to monophosphate form (dFdCMP) and then phosphorylated again by pyrimidine nucleoside monophosphate kinase (UMPCMP kinase) to gemcitabine diphosphate [10]. Gemcitabine is also phosphorylated by a mitochondrial enzyme, thymidine kinase 2 (TK2), which plays a role in phosphorylation of natural nucleosides. The substrate specificity of this enzyme for gemcitabine is, however, only 5–10% when compared to deoxycytidine. Thus, phosphorylation of gemcitabine by dCK to active metabolite is the rate limiting step for further phosphorylation of this compound, which is essential for its cytotoxic activity. DCK deficiency is also described in acquired and intrinsic resistance to gemcitabine in some in vitro and in vivo experiments [11].

Gemcitabine may become inactivated through deamination by cytidine deaminase (CDA). In particular, CDA has lower affinity for gemcitabine in comparison with deoxycytidine. The gemcitabine deamination product is 2′,2′-difluoro-2′-deoxyuridine (dFdU), which has several intracellular regulatory roles in transportation, accumulation and cytotoxic effect of gemcitabine [12].

Gemcitabine can also become inactivated by dephosphorylation of monophosphate form by 5′-nucleotidases ('5-NTs), converting nucleotides back to nucleosides [9].

1.3. Effect of gemcitabine on cellular process leading to apoptosis

The most important effect of gemcitabine is inhibition of DNA synthesis. When dFdCTP is incorporated into DNA, another single deoxynucleotide is incorporated afterwards, halting chain elongation. Presence of this additional base pair before termination prevents from its detection by DNA repair system, which is known as “masked chain termination”. This process also inhibits the removal of gemcitabine by DNA repair enzymes [13].

Metabolites of dFdC, like dFdCTP, are also assumed to be inserted into RNA. The effect of this RNA insertion on cell function is not elucidated yet. Keeping the concentrations of di- and triphosphate forms of gemcitabine at high level provides for successful incorporation of the drug into nucleic acids via reducing the competing deoxyribonucleotide pools necessary for DNA and RNA synthesis (self-potentiation) [9,10].

The potentiation effect of 2′,2′-difluoro-2′-deoxycytidine triphosphate (dFdCTP) results from the inhibition of ribonucleotide reductase(s) (RNR) enzyme, which generates deoxynucleoside 5′-diphosphates (dNDPs) from nucleoside diphosphates. Ribonucleotide reductase consists of two subunits: ribonucleotide reductase M1 (RRM1) and ribonucleotide reductase M2 (RRM2). RNRs can be inhibited by F2CDP, which leads to reduction in deoxy-dinucleotide (dNDP) pools consequently leading to reduction in dNTP pools. Reduced concentrations of dNTPs diminish the competition for F2CTP to be incorporated into DNA by DNA polymerase [10,14,15], thereby preventing cellular growth and initiating apoptosis (Fig. 1) [9].

Activation of caspase signaling pathway is also another important mechanism of apoptosis induced by gemcitabine. Gemcitabine

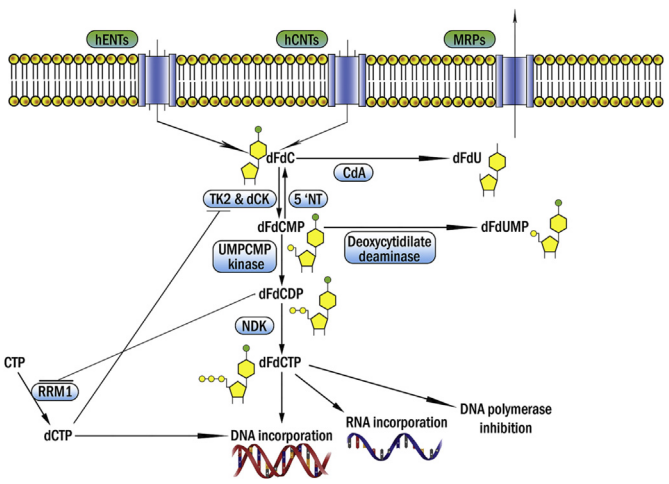

Fig. 1. Gemcitabine pharmacologic pathway representing drug transport, metabolism mechanisms of action and self-potentiation. Transport by nucleotide transporters (hNTs); phosphorylation and deamination; dephosphorylation; accumulation of the triphosphate; incorporation into DNA; incorporation into RNA; inhibition of ribonucleotide reductase (RR); inhibition of CTP-synthase; inhibition of thymidylate synthase (TS); inhibition of deoxycytidine monophosphate deaminase (dCMPDA). Other abbreviation include dCK: deoxycytidine kinase; TK2: thymidine kinase2; dCDA: desoxycytidine deaminase; 5′-NT: 5′-nucleotidase.

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