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Review

Gemcitabine: Metabolism and molecular mechanisms of action, sensitivity and chemoresistance in pancreatic cancer

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ABSTRACT

Gemcitabine is the first-line treatment for pancreatic adenocarcinoma, but is increasingly used to treat breast, bladder, and non-small cell lung cancers. Despite such broad use, intrinsic and acquired chemoresistance is common. In general, the underlying mechanisms of chemoresistance are poorly understood. Here, current knowledge of gemcitabine metabolism, mechanisms of action, sensitivity and chemoresistance reported over the past two decades are reviewed; and we also offer new perspectives to improve gemcitabine efficacy with particular reference to the treatment of pancreatic cancer.

Contents

Abbreviations: 5′-NT, 5′-nucleotidase; APE1/Ref1, Apurinic/apyrimidinic endonuclease 1/redox-factor-1; CDA, Cytidine deaminase; cN, Cytosolic 5′-nucleotidase; CXCL12, C-X-C motif chemokine 12; CXCR4, C-X-C motif chemokine receptor 4; dCK, Deoxycytidine kinase; dCTD, Deoxycytidylate deaminase; dFdC, 2′,2′-difluoro-2′-deoxycytidine (gemcitabine); dFdCDP, 2′,2′-difluoro-2′-deoxycytidine diphosphate (gemcitabine diphosphate); dFdCMP, 2′,2′-difluoro-2′-deoxycytidine monophosphate (gemcitabine monophosphate); dFdCTP, 2′,2′-difluoro-2′-deoxycytidine triphosphate (gemcitabine triphosphate); dFdU, 2′,2′-difluoro-2′-deoxyuridine; dFdUMP, 2′,2′-difluoro-2′-deoxyuridine monophosphate; dNTP, Deoxynucleotide triphosphate; ERK, extracellular-signal regulated kinase; FAK, focal adhesion kinase; FDA, US Food and Drug Administration; FOLFIRINOX, leucovorin, 5-fluorouracil, irinotecan and oxaliplatin; hCNT, Human Concentrative Nucleoside Transporter; hENT, Human Equilibrative Nucleoside Transporter; Hh, Hedgehog signalling; HIF-1α, hypoxia inducible factor -1α; HMGA1, High mobility group A1; hNT, Human Nucleoside Transporter; Hsp27, Heat shock protein 27; HuR, Hu antigen R; IFN-γ, interferon gamma; IL-1β, interleukin-1β; KRAS, V-Kirsten rat sarcoma viral oncogene homolog; MAPK, Mitogen-activated protein kinase; MUC4, mucin 4; NF-κB, nuclear factor-κB; NGS, next-generation sequencing; PI3K, phosphatidylinositol 3-kinase; RR, Ribonucleotide Reductase; RRM1, Ribonucleotide Reductase subunit I; RRM2, Ribonucleotide Reductase subunit II; Shh, Sonic Hedgehog; TNFα, tumor necrosis factor α; TP53, tumor suppressor protein 53; UBE2M, Ubiquitin-conjugating enzyme E2M; UMP-CMP kinase, Pyrimidine nucleoside monophosphate kinase

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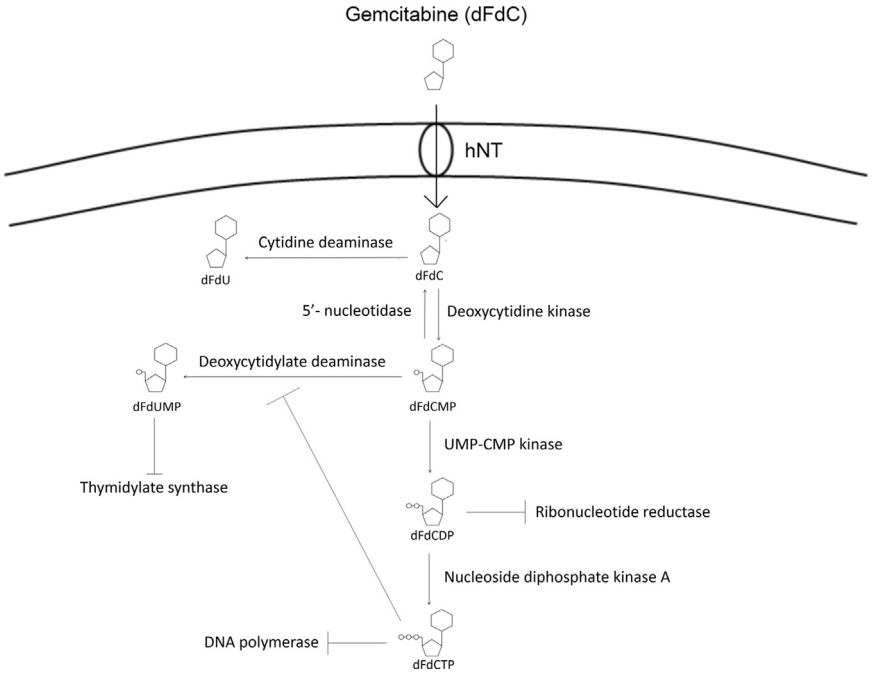

Fig. 1. Gemcitabine cellular metabolism. hNT: human nucleoside transporter; dFdCMP: gemcitabine monophosphate; dFdCDP: gemcitabine diphosphate; dFdCTP: gemcitabine triphosphate; dFdU: 2′,2′-difluoro-2′-deoxyuridine, dFdUMP: 2′,2′-difluoro-2′-deoxyuridine monophosphate.

1. Introduction

Gemcitabine is a nucleoside analog that has been used as a chemotherapeutic agent for more than 15 years. During this time gemcitabine has become the standard treatment choice for locally advanced and metastatic pancreatic cancer, for which there have been few treatment advances. Pancreatic cancer is one of the most devastating human cancers, with a median survival rate of less than 5 months after diagnosis and with under a 5% five-year survival rate even if brought under remission (Bilimoria et al., 2007). This poor prognosis is partly explained because of poor penetration of drugs into the dense and under vascularized tumour stroma (Neesse et al., 2011), a high degree of acquired chemoresistance by tumour cells and also because surgical intervention is not possible in almost 80% of patients (Vincent et al., 2011).

Gemcitabine is also used to treat a variety of solid tumors including breast, ovarian, and non-small cell lung cancer, especially when in combination with the platinum-based drugs cisplatin and carboplatin (Nagourney et al., 2008; Pfisterer et al., 2006; Reck et al., 2009). Despite its relatively broad and common use, mechanisms related to gemcitabine resistance are not well understood. A number of different cellular pathways, transcriptional factors and nucleotide metabolism enzymes have been linked to gemcitabine resistance and sensitivity.

Here the known molecular mechanisms commonly associated with gemcitabine pharmacokinetics and pharmacodynamics are reviewed with a special focus on pancreatic cancer. A better understanding of gemcitabine pharmacology is essential for developing improved treatments and ultimately for increasing patient survival in pancreatic cancer.

2. Uptake and metabolism

Gemcitabine (2′,2′-difluoro-2′-deoxycytidine; dFdC) is a deoxycytidine analog with multiple modes of action inside the cell. The basics of gemcitabine metabolism are illustrated in Fig. 1. As a prodrug, dFdC must be metabolized to the active triphosphate form of gemcitabine (2′,2′-difluoro-2′-deoxycytidine triphosphate; dFdCTP). 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 the hydrophilic nature of nucleosides and nucleoside analogs (Mackey et al., 1998). Two types of hNTs are recognized that differ according to mechanisms of transport called equilibrative and concentrative nucleoside transporters (hENT and hCNT, respectively) (Spratlin et al., 2004). Four hENTs (hENT1, hENT2, hENT3, and hENT4) and three hCNTs (hCNT1, hCNT2, and hCNT3) have been identified (Mackey et al., 1998). Amongst these, it has been demonstrated that the majority of gemcitabine uptake is mediated by hENT1 and, to a lesser extent, also by hENT2, hCNT1 and hCNT3 (Mackey et al., 1998; Ritzel et al., 2001).

Once inside the cell, gemcitabine is phosphorylated in the cytoplasm by deoxycytidine kinase (dCK) to the monophosphate (dFdCMP) and then phosphorylated again by pyrimidine nucleoside monophosphate kinase (UMP-CMP kinase) to give gemcitabine diphosphate (dFdCDP) (Heinemann et al., 1988; Bouffard et al., 1993; Hatzis et al., 1998; Van Rompay et al., 1999). Other, as yet unknown enzymes might also perform the second phosphorylation step, as Hu et al. have reported that down- or up-regulation of UMP-CMP kinase does not effect the levels of dFdCDP and dFdCTP in human colorectal carcinoma cells (Hu et al., 2011). The enzyme responsible for the final phosphorylation step (dFdCDP into the active metabolite dFdCTP) is unclear, although nucleoside diphosphate kinase may play this role (Wong et al., 2009). The first phosphorylation by dCK is considered the rate-limiting step for dFdCDP and dFdCTP production (Ruiz van Haperen et al., 1996; Ohhashi et al., 2008).

Gemcitabine may become inactivated through deamination by cytidine deaminase (CDA) and, when in the monophosphate form by deoxycytidylate deaminase (dCTD) (Heinemann et al., 1992; Xu and Plunkett, 1992). Notably, CDA has nearly half of the affinity for gemcitabine in comparison with deoxycytidine (Bouffard et al., 1993). The product of gemcitabine deamination by CDA is 2′,2′-difluoro-2′-deoxyuridine (dFdU), which has several postulated