



Potential Mechanism of Chemo-Resistance to Gemcitabine



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Abstract

Gemcitabine (2',2'-difluorodeoxycytidine, Gemzar®) is a deoxycytidine analog with pronounced antitumor activity against a variety of solid tumors. As gemcitabine is widely used for the treatment of human cancer, the appearance of resistance is a major obstacle to its clinical efficacy. Therefore, it is very important to understand the mechanisms by which cancer cells evolve chemo-resistance to this drug. Through comprehensive analysis of gene -expression profiles by oligonucleotide microarray analysis and follow -up studies, we have identified a cluster of DNA repair genes whose expression is induced in MDA- MB231 cells in the presence of gemcitabine. Based upon information obtained from this analysis, a testable hypothesis was generated regarding the potential roles of identified DNA repair gene products in mediating chemo resistance to gemcitabine in human cancer. Furthermore, new insights can be gained into the potential signaling pathways that interface the DNA damage checkpoint pathways with the activation of DNA repair processes by up regulating a selected group of DNA repair genes in cancer cells treated with gemcitabine.

Keywords: Gemcitabine; DNA repair; Chemoresistance; DNA damage checkpoint

Abbreviations: Ara-C: Cytosine Arabinoside; ATM: Ataxia-Telangiectasia-Mutated; ATR: Ataxia Telangiectasia and Rad3-related; Chk1: Checkpoint Kinases 1, dCK: Deoxycytidine Kinase; RRM1: Ribonucleotide Reductase M1; RRM2: Ribonucleotide Reductase M2

Introduction

Gemcitabine (2',2'-difluoro 2'-deoxycytidine, dFdC) is the most important cytidine analogue developed since cytosine arabinoside (Ara-C) [1-2]. Influx of gemcitabine through the cell membrane is believed to be mediated by nucleoside transporters (hENT1). Gemcitabine inside the cells is subsequently converted to gemcitabine diphosphate (dFdCDP) and triphosphate (dFdCTP) by deoxycytidine kinase (dCK) [3]. dFdCDP is a potent inhibitor of ribonucleotide reductase, lowering dNTP pools required for DNA synthesis, subsequently potentiating the effects of dFdCTP [3]. dFdCTP is a potent inhibitor of DNA polymerase as a competing substrate of dCTP [4-7]. Incorporation of dFdCTP into DNA by DNA polymerase results in DNA strand termination, arresting of replication forks [4]. Such DNA replication blocking activities are known to potentiate major DNA damage, leading to genomic instability. DNA replication fork stalling at a DNA lesion subsequently can activate DNA damage checkpoints [8,9]. Previous studies demonstrated that the ATR/Chk1 signaling pathway is known to promote cell survival by blocking additional origin firing and stabilizing stalled replication forks in response to replication stress caused by gemcitabine, [8,9]. Although the ATM pathway has typically been implicated in the response to DNA double-strand breaks rather than nucleoside analogs, it was recently found that ATM also plays an important

role in gemcitabine-treated cells [10]. Mammalian cells mount a coordinated response to DNA damage, activating DNA damage checkpoints and an entire ensemble of cellular responses to DNA damage, including: [1] an arrest of cell cycle progression, allowing for repair and prevention of the replication of damaged or incompletely replicated chromosomes; [2] a transcriptional response, which causes changes in the transcription profile that results in removal of DNA damage and restoration of the continuity of the DNA duplex; and [3] apoptosis, which eliminates heavily damaged or seriously deregulated cells [11-14].

Gemcitabine is structurally and pharmacologically similar to Ara-C, but also displays distinctive features of cellular pharmacology, metabolism, and mechanism of action [15-17]. Gemcitabine resistance has been thought to be mainly associated with decreased expression of hENT1, a deficiency in dCK activity, or an increase in ribonucleotide reductase M1 (RRM1) and M2 (RRM2) activity [18-21]. More recently, acquired resistance to gemcitabine is proposed to be associated with four proteins involved in gemcitabine transport and metabolism, including hENT1, dCK, RRM1 and RRM2 in pancreatic cancer patients [22]. Gemcitabine resistance is also associated with many other factors, such as hypoxia, deregulation of apoptotic proteins (e.g.,

Bcl-2, Bax, and Bak), NF- κ B, and phosphatidylinositol kinase/Akt survival pathways [23]. Additionally, increased expression and activation of focal adhesion kinase, c-Src, and c-Met have been reported to be implicated in gemcitabine resistance [23].

Despite the widespread use of gemcitabine, survival benefit and clinical impact remains modest due to a high degree of intrinsic and acquired resistance [24,25]. Therefore, further investigation into the mechanisms of its activation and development of resistance against it is required. Since gemcitabine also acts on DNA and alters the structure of the DNA molecule [4-7], the role that DNA repair pathways play in mediating gemcitabine resistance has been studied for many years in both preclinical and clinical settings with the expectation that this knowledge can be prospectively used in the clinical arena [26-28]. We hypothesized that cancer cells exposed to gemcitabine would up regulate the DNA repair genes, increasing the capability of the cancer cells to detect and subsequently correct the DNA damage caused by gemcitabine. Our hypothesis was based on previous observations made in our and other laboratories that cancer cells exposed to DNA damaging agents or radiation are often known to transiently induce DNA repair genes, thereby increasing the capacity of these cells to repair DNA lesions [29-36]. This can be explained partly because the constitutive elevation of certain repair proteins may even prove detrimental to the cells by causing endogenous damage to DNA [37,38]. This is particularly clear for PARP-1, whose imbalanced production leads to necrotic cell death as a result of NAD⁺ and ATP depletion [39]. Therefore, it can be assumed that cancer cells might become resistant to DNA damaging anticancer agents, such as gemcitabine by transiently upregulating DNA repair genes encoding DNA repair proteins in response to DNA damage without the burden of constitutively over expressing certain DNA repair proteins that would have adverse effects on long-term survival.

Recently, the expression patterns of DNA repair genes in gemcitabine-treated cancer cells (e.g., MDA-MB231) were analyzed by using DNA arrays from Agilent Technologies (Whole Human Genome Oligo Microarray 44K) with the expectation of identifying gemcitabine-inducible DNA repair genes whose gene products might play a central role in repairing DNA damage occurring after exposure to gemcitabine. Our preliminary studies suggest that many genes whose functions are implicated in DNA repair pathways were transcriptionally induced after gemcitabine treatment of cancer cells. Interestingly, our preliminary data also suggest that the mechanism of this induction involves the activation of DNA damage checkpoint pathways, providing the first evidence for the transcriptional activation of a group of DNA repair genes in response to the activation of DNA damage checkpoint pathways [40]. Our data offers an early glimpse of potential mechanism of chemo resistance to gemcitabine; alterations in the expression of these DNA repair genes impact the cellular response to gemcitabine. However, more studies will be necessary to clarify if the induction of DNA repair genes after

gemcitabine exposure could confer resistance to gemcitabine in tumor cells and to determine if the modulation of this activity by pharmacological or genetic approaches could have therapeutic potential when combined with gemcitabine treatment.

Conclusion

In future studies, the biological significance of the induction of select DNA repair genes responding to gemcitabine treatment in human cancer cells should be characterized. In particular, it should be addressed whether the induction of DNA repair genes in gemcitabine-treated cancer cells is associated with chemo resistance to gemcitabine. It is also important to identify the potential signal transduction pathways involved in mediating the induction of DNA repair genes in cancer cells after exposure to gemcitabine. There might be unidentified components interconnecting DNA damage checkpoints and the activation of DNA repair activity in response to DNA damage and replication stress caused by gemcitabine. To obtain more clinically relevant data, it is important to investigate whether the expression

of candidate DNA repair genes is elevated in gemcitabine-treated tumors relative to untreated control tumors using in vivo tumor models. The investigation of DNA repair proteins or pathways that are more selectively and consistently induced by gemcitabine in cancer cells than in normal cells could lead to the development of potential strategies to maximize the therapeutic index of gemcitabine in the future. Eventually, new agents can be evolved, which exert anticancer effects through their ability to interfere with DNA repair proteins selectively induced by gemcitabine treatment.

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