Gemcitabine Resistance in Pancreatic Cancer: Picking the Key Players

□□ *Commentary on Liau and Whang, p. 1470*

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The diagnosis of pancreatic cancer confers a grim prognosis that has changed little over the last 30 years. Due to the lack of any efficacious screening or biomarkers for pancreatic cancer, presentation with advanced local disease is typical (classically, painless jaundice) and morbidity aside, 4% of patients are alive 5 years after the time of diagnosis. The nucleoside analogue gemcitabine, which today remains the cornerstone of neoadjuvant and adjuvant chemotherapy in pancreatic cancer, has only a 5.4% partial response rate (1) and imparts a progression-free survival interval ranging from 0.9 to 4.2 months (2). However, many forms of pancreatic cancer show initial sensitivity to gemcitabine therapy followed by the rapid development of resistance—a feature that essentially characterizes this fatal disease. Although the rapid acquisition of resistance to gemcitabine treatment inevitably translates into poor patient outcomes, the tumor's initial vulnerability and subsequent resistance strongly suggest either the preexistence of resistant cell subpopulation(s) or the rapid development of resistant cells from the tumor itself or from tumor/stromal alterations. Thus, a better understanding of the origins of gemcitabine resistance is critical to the development of superior combination therapies or the replacement of gemcitabine as the gold standard in pancreatic cancer.

Several genetic and/or epigenetic alterations have been associated with gemcitabine resistance. Not surprisingly, these include gene products associated with gemcitabine transport and metabolism. Alterations in the nucleoside transporter-1 (hENT1), an important element in gemcitabine uptake, as well as various gemcitabine metabolism gene products, including deoxycytidine kinase and ribonucleoside reductases M1 and M2, have been shown in gemcitabine resistance (3–6). Aberrant expression of genes associated with cellular survival and apoptosis have been implicated, such as the S100 family member S100A4, the expression of which may increase resistance in part by regulation of the hypoxia-induced proapoptotic gene *BNIP3* (7, 8). The phosphatidylinositol 3-kinase/Akt survival pathway has also been implicated in gemcitabine resistance (9–12) along with integrin-linked kinase (13). Increased expression and activation of the non–receptor protein tyrosine kinases focal adhesion kinase (14) and c-Src (15, 16) have both been associated with gemcitabine resistance. Finally, c-Met activation has also been implicated in gemcitabine resistance (17).

In this issue of *Clinical Cancer Research*, Liau and Whang extend their previous work (18) demonstrating that expression of HMGA1, a transcriptional "enhanceosome," plays a role in sensitivity/resistance of pancreatic tumor cells to gemcitabine both *in vitro* and in mouse xenograft models (19). Overexpression of HMGA1 has been implicated in a number of human cancers and may correlate with poor prognosis (20, 21). HMGA1 complexes on chromatin regulate transcription of numerous genes downstream of the Ras/extracellular signal-regulated kinase signaling pathway. *HMGA1* itself is a target gene of c-Myc, c-Jun, and activator protein-1 (22–24). Thus, this protein is associated with numerous functions that could be important in drug resistance. Building upon their previous *in vitro* study that implicated HMGA1 in the sensitization of the pancreatic cancer cell lines BxPC3 and MiaPcCa2 (18), in this work the authors show that decreased HMGA1 expression by RNA interference techniques increases apoptosis and sensitizes cells to gemcitabine *in vitro*, and also results in decreased tumor burden upon gemcitabine treatment in subcutaneous xenografts in nude mice. Similar sensitization occurred with a dominant negative Akt construct, suggesting that HMGA1 signals through the prosurvival Akt pathway. These studies once again reaffirm the importance of activation of the Akt pathway in drug resistance.

The most important finding in the work by Liau and Whang is the effect of gemcitabine treatment on HMGA1 knockdown cells *in vivo*. Although less characterized than MiaPaCa2 cells, gemcitabine administration in mice bearing subcutaneous xenografts of BxPC3 cells, in which HMGA1 was down-regulated, led to tumor regression, whereas parental cells continued to grow. As with *in vitro* studies, increased apoptosis was associated with tumor regression *in vivo*, although involvement of the Akt pathway was not determined. Although further investigation is needed to validate HMGA1 as therapeutic target, Liau and Whang's results are exciting because they suggest that it may be feasible to identify "gemcitabine-sensitizing agents"—drugs that attenuate inherent and acquired resistance to gemcitabine. As of yet, we have little idea what transcriptional program(s) are regulated by HMGA1 or how HMGA1 overexpression leads to Akt activation. Consequently, studies in these areas will be required to determine whether strategies to suppress HMGA1 activity will translate to clinical application.

An important consideration in the study of gemcitabine resistance in pancreatic cancer is the apparent phenotypic and molecular variability among gemcitabine-resistant models isolated to date, as well as the large number of gene products

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that ostensibly regulate gemcitabine resistance. As most reports of gemcitabine resistance have taken a reductionist approach, examining individual genes in a limited number of pancreatic cancer cell lines, it remains uncertain whether resistance might result from changes in expression of specific "key" genes or if gemcitabine treatment is most frequently countered by common and predictable epigenetic or genetic programs. Thus, current studies raise several fundamental questions. If altered expression of multiple individual gene products is sufficient to induce resistance, have we only identified a fraction of the genes, the aberrant expression of which might lead to gemcitabine resistance? If so, the therapeutic implications of gemcitabine-sensitizing targets and agents are daunting. Use of such agents would likely be tailored on a patient-by-patient basis, using specific sensitizers based on the characterization of resistance mechanisms for individual tumors. Also, is it possible that our findings to date are simply artifacts of *in vitro* manipulation? Additional comparisons of resistance in cell line models to changes in gene expression in "fresh" pancreatic tumors are required to answer this latter question.

However, a more optimistic possibility is that targeted therapy aimed at critical, common elements in resistance profiles will restore sensitivity. For example, others and we have noted that nucleoside resistance is associated with both a more mesenchymal phenotype and an increase in stem cell markers (25, 26). Such phenotypes seem common in a variety of solid tumors with respect to resistance to other chemotherapeutic agents. Should these observations prove general, they offer new approaches to target select gene products and overcome the serious clinical problem of rapid resistance to gemcitabine treatment in pancreatic tumor cells. Regardless, the identification of gene products that affect gemcitabine sensitivity/resistance, such as HMGA1, is a fundamental step forward in understanding the key molecular players that promote the acquisition of deadly cancer phenotypes.

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