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Research Article

Molecular Cancer Therapeutics

A Fine-Needle Aspirate–Based Vulnerability Assay Identifies Polo-Like Kinase 1 as a Mediator of Gemcitabine Resistance in Pancreatic Cancer

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Abstract

This work aimed to discover targets for combination treatment with gemcitabine in pancreatic cancer. We selected 11 tumors from our live collection of freshly generated pancreatic cancer xenografts with known degrees of varying gemcitabine sensitivity. We briefly (6 h) exposed fine-needle aspiration material to control vehicle or gemcitabine (1 μmol/L) and compared the gene expression of the treated and untreated samples using a reverse transcription-PCR–based, customized low-density array with 45 target genes of therapeutic interest. The gene expression of the untreated sample (which can be considered a baseline/static readout) was not predictive of gemcitabine efficacy in these tumors. Altogether, the only gene that differentiated sensitive versus resistant cases was polo-like kinase 1 (Plk1), showing >50% downregulation in sensitive cases and no change in the resistant cases. Inhibition of Plk1 by either small interfering RNA gene knockdown or with the Plk1 pathway modulator (ON 01910.Na) synergized with gemcitabine in gemcitabine-refractory *in vitro* models providing mechanistic proof of concept. *In vivo* experiments in gemcitabine-resistant xenografts showed synergistic activity decreasing cell proliferation and tumor regressions. A quantitative gene expression–based vulnerability assay identified Plk1 as a relevant target dictating the susceptibility of pancreatic cancer to gemcitabine. Dynamic interrogation of cancer has the potential to provide key information about mechanisms of resistance and to enhance individualization of treatment. *Mol Cancer Ther; 9(2); 311–8. ©2010 AACR.*

Introduction

Pancreatic cancer remains a devastating disease as shown by the rough equivalence of incidence and mortality rates (1). At the time of diagnosis, 80% of patients have locally advanced or advanced disease for which no curative therapy exist, and 80% of patients treated with curative intent will recur in the first 2 years after surgical resection and will succumb to their disease (2). Gemcitabine is the only agent that has been convincingly shown to be efficacious but has a limited activity, and virtually all patients show either primary or ultimately acquired resistance. The factors determining gemcitabine resistance are currently unknown. Also, the vast majority of studies that tested a combination of an agent with gemcitabine versus gemcitabine alone have failed, with the common denominator that the combination agent was chosen empirically.

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Although there are many new agents entering clinical development, often there is no information on biomarkers that may predict the activity of these drugs. Our group has develop an *in vivo* model of pancreatic cancer implanting tumor material obtained at the time of surgical resection in nude mice (3). In this work, we aimed at rationally identifying potential targets for combination therapy with gemcitabine in pancreatic cancer by using a dynamic *ex vivo* assay. In previously reported work (4), we have optimized the use of fine-needle aspirate (FNA) biopsies as a platform to conduct *ex vivo* predictive assays that have been optimized in a clinically applicable manner. Currently, there are no grounds to prioritize which agents should be administered to a given patient with pancreatic cancer. Sensitive and resistance assays have addressed this issue but have largely failed as they are based on clonogenicity and/or proliferation indexes and require both acquiring large amounts of tissue and maintaining cell viability for extended periods (5, 6). On the other hand, it is possible to elicit pharmacodynamic responses by briefly exposing small amounts of tumor cells to a drug, an approach termed *ex vivo* testing (7).

There is increased awareness of the complexity of pancreatic cancer, and it is unlikely that single-gene alterations will help design therapies for broad subsets of patients (8). We have hypothesized that interrogating the integrative response of a complex system to a pharmacologic insult

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will provide valuable information. Here, we aimed at rationally identifying relevant targets involved in the vulnerability of pancreatic cancer to gemcitabine. We dynamically interrogated a set of pancreatic samples with known gemcitabine sensitivity by exposing them to gemcitabine and analyzing the early response of a preselected set of relevant drug target genes.

Materials and Methods

Drugs

Gemcitabine (Elli Lilly) was dissolved in PBS. ON 01910.Na was obtained from Onconova Therapeutics and dissolved in PBS.

Cell Lines and *In vitro* Culture Conditions

The cell line MiaPaca2 was obtained from the American Tissue Culture Collection and derived from a pancreatic carcinoma. The cell lines Panc813 and Panc1005 are low-passage pancreatic cell lines and were a kind gift from Dr. Elizabeth Jaffee. The cell lines XPa3 and XPa4 are low-passage pancreatic cell lines and were a kind gift from Dr. Anirban Maitra. The cell line E3JD13 is a low-passage pancreatic cell line obtained from a rapid autopsy program and was a kind gift from Dr. Christine Iacobuzio-Donahue. For mRNA and protein expression analysis, the cell lines were grown in six-well plates. When the experiments ended, they were washed twice with chilled PBS, lysis buffer or RLT (Qiagen) was added to the plates, and cells were scraped for protein or mRNA extraction.

In vivo Growth Inhibition Studies

Six-week-old female athymic nude mice (Harlan) were used. The research protocol was approved by the Johns Hopkins University Animal Care and Use Committee, and animals were maintained in accordance to guidelines of the American Association of Laboratory Animal Care. The xenografts were generated according to methodology published elsewhere (3). Tumors are kept as a live bank that is expanded as required for drug testing and biological studies. In the initial gemcitabine experiments, tumors from 11 patient cases (253, 215, JH021, JH011, JH015, 163, JH027, 410, 219, JH033, and 286) were allowed to grow until reaching ~200 mm^3, at which time mice were distributed in the following two treatment groups, with 5 to 6 mice (10 evaluable tumors) in each group: control (vehicle) and gemcitabine (100 mg/kg i.p. twice a week for 28 days). In the subsequent experiments, tumors from 3 patient cases (410, JH033, and 286) were allowed to grow until reaching ~200 mm^3, at which time mice were distributed in the following four treatment groups, with 5 to 6 mice (10 evaluable tumors) in each group: control (vehicle), gemcitabine (100 mg/kg i.p. twice a week), ON 01910.Na (250 mg/kg i.p. five times a week for 28 days), and the combination of both drugs at the same doses. Tumor size was evaluated two times a week by caliper measurements using the following formula: tumor volume = (length × $width^2$)/2. Relative tumor growth inhibition was calculated by relative tumor growth of treated mice divided by relative tumor growth of control mice since the initiation of therapy (T/C).

FNA and *Ex vivo* Rapid Molecular Assay

FNA biopsies were done according to standard cytopathologic practice under inhaled general anesthesia (isofluorane) using 10 cc syringes and 25-gauge needles. During each FNA procedure, the first pass was smeared onto glass slides and used for morphologic analysis (Diff-Quik and Papanicoloau), and three to four subsequent passes were used for viable cell harvesting. We acquired the passes from separate parts of the tumor to avoid contamination and bleeding and to sample each tumor comprehensively. The material collected by three to four FNA passes on untreated tumors derived from the 11 cases from direct pancreatic cancer xenografts was aliquoted in growth medium, seeded in six-well plates, and treated for 6 h with growth medium and growth medium plus gemcitabine at a concentration of 1 μmol/L. The material was resuspended, washed twice with chilled PBS, and lysed with RLT for RNA evaluation.

Low-Density Microarrays

The untreated and treated samples from the *ex vivo* assay of each case were run in a customized assay (ABI). This platform consists of a multichannel 384-well plate that is distributed in four sections, each formed by 96 wells precoated with the primers corresponding to 48 selected genes. Three such genes corresponded to housekeeping genes, and the other 45 were selected based on ad hoc criteria (not all needed to apply for the gene to be selected for inclusion): (*a*) regulated at mRNA level, (*b*) relevant for pancreatic cancer, and (*c*) inhibitor in existence or development.

Small Interfering RNA

Polo-like kinase 1 (Plk1) small interfering RNA (siRNA) and transfection reagent (Dharmacon RNA Technologies) were optimized in 24-well cell culture plates where 30,000 cells were added to each well in 500 μL growth medium and grown overnight at 37°C. Each plate contained wells for antibiotic-free medium alone, wells for 0.2%, 0.4%, and 0.6% of transfection reagent to final volume with antibiotic-free medium, and wells for the varying transfection reagent amount plus 100 and 200 nmol/L siRNA concentrations in antibiotic-free medium. Conditions were tested for 24 and 48 h. Cells were harvested with RLT buffer (Qiagen). After optimization of siRNA conditions in all six cell lines, the optimal concentration of transfection reagent, siRNA, and time were 0.4%, 200 nmol/L, and 48 h, respectively.

In vitro Endpoint Testing

In vitro drug sensitivity to gemcitabine, ON 01910.Na, and the combination of both (all at concentrations of 1 μmol/L for 72 h) was assessed by MTT (Sigma). For the MTT-based efficacy studies after siRNA, cells were seeded in 96-well plates, transfected during 48 h with the above conditions, and then exposed to either vehicle

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or gemcitabine at 1 μmol/L for 72 h. Each experiment was done in sextuplicate and was carried out independently at least three times.

Quantitative Real-time Reverse Transcription-PCR Analysis

Total RNA was extracted from *ex vivo* material, cell pellets, and tumors using the RNeasy Mini kit (Qiagen). cDNA was synthesized using iScript cDNA synthesis kit (Bio-Rad) following the manufacturer's instructions. Relative quantification of Plk1 and ubiquitin C (used as a housekeeping gene) mRNA was achieved using an iCycler iQ real-time PCR detection system (Bio-Rad) using ABI Taqman probes. The experiments were repeated twice, and samples were run in quadruplicate.

Immunohistochemical Analysis

Sections (5 μm) were used for cyclin B1 and Ki-67 staining. After rehydrating, antigen retrieval was done using a Target Retrieval Solution (DAKO) heated for 40 min. Endogenous peroxidase activity was quenched by incubation in 3% hydrogen peroxide. Next, the cyclin B1 primary antibody (eBioscience) was diluted 1:100 and incubated at room temperature for 1 h followed by an anti-rabbit secondary antibody (Envision$^+$; DAKO) for 30 min and 3,3′-diaminobenzidine (K3468; DAKO) for 5 min. Between steps, slides were washed in PBS with 1% Tween 20. Four tumors per treatment group were blindly analyzed. For statistical analyses, an index of staining intensity × percentage of cells staining positive was calculated.

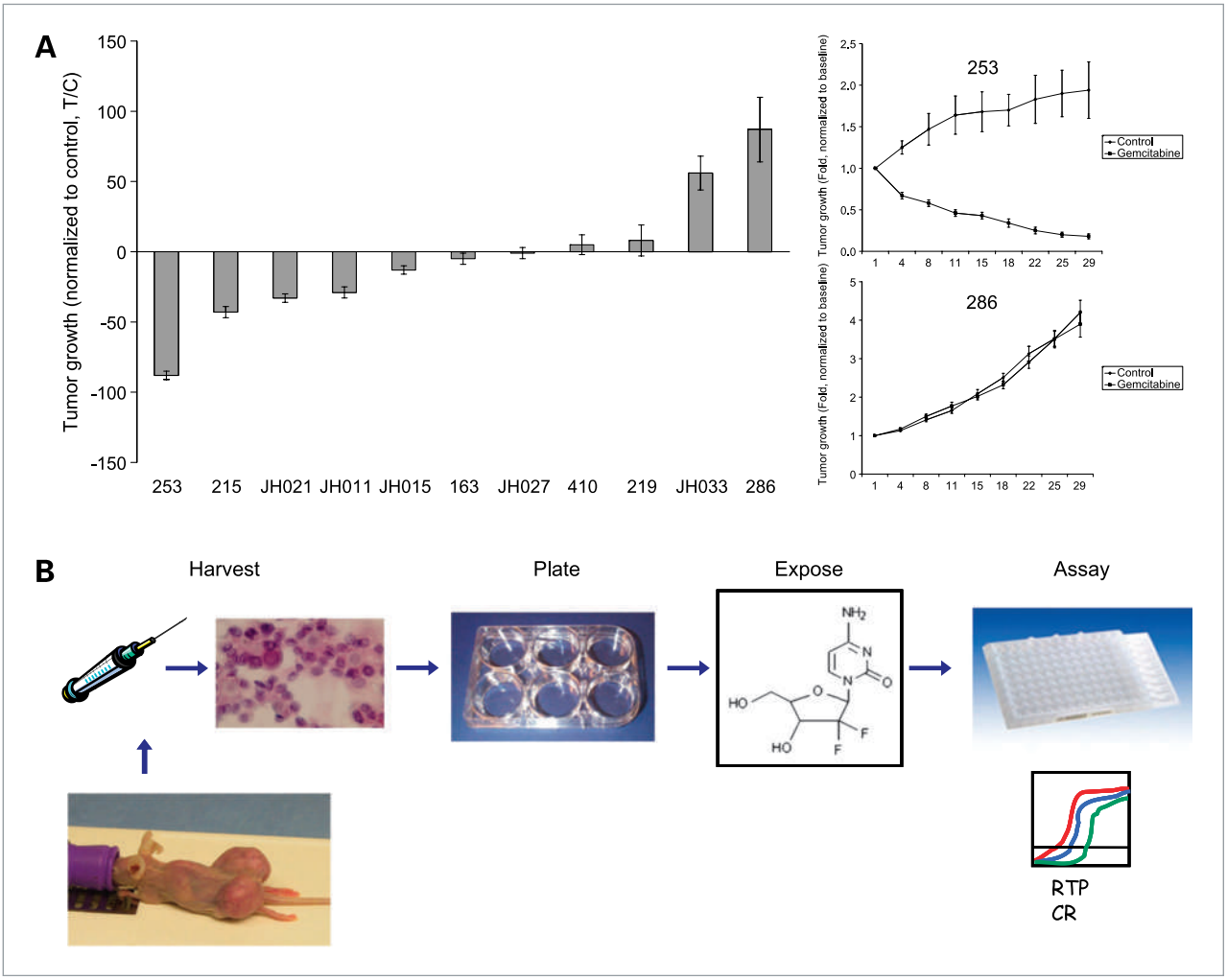

Figure 1. A, gemcitabine sensitivity of 11 direct xenograft cases. Each column is normalized to the untreated control. A negative value indicates that tumor size at the completion of the experiment was smaller than at initiation, indicating tumor regression. See two illustrative examples on the right, with the experimental plots of the most sensitive and most resistant cases 253 and 286, respectively. Bars, SE (n = 10 tumors per group). B, *ex vivo* assay: a small sample of tissue is acquired by FNA biopsy from a tumor xenografted on mice and is plated and exposed to gemcitabine for 6 h. RNA is then collected and analyzed by reverse transcription-PCR in the low-density array platform.

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Table 1. Low-density array of FNA-acquired samples exposed *ex vivo*

	Sensitive									Resistant	
Gem T/C	−88	−43	−33	−29	−13	−5	−1	5	8	56	87
Case	253	215	JH021	JH011	JH015	163	JH027	410	219	JH033	286
CyclinB1	99	52	124	68	84	94	53	16	140	89	115
TGFA	113	92	85	99	101	109	97	131	107	141	103
HIF1A	111	106	146	126	102	113	108	115	113	90	100
Survivin	110	58	166	81	145	84	67	85	74	64	104
AXIN2	63	44	142	67	517	248	210	41	81	202	106
MSLN	118	91	89	101	101	99	100	117	98	90	107
FOS	115	123	108	113	106	111	112	139	113	96	83
BRCA1	63	84	343	81	200	114	95	89	59	62	161
RRM1	166	81	79	110	114	91	92	129	104	92	73
BRAF	98	93	175	116	88	88	146	40	122	65	93
GADD45A	108	104	78	108	75	89	121	96	147	106	74
SHH	108	65	91	70	116	66	113	57	77	70	47
NFKB1	138	94	104	114	83	91	163	105	105	78	110
p21	110	99	102	96	122	84	143	134	100	119	83
BNIP3L	96	82	115	109	105	92	90	100	106	97	95
NOTCH2	119	103	92	113	79	81	56	112	122	101	101
VHL	116	118	127	104	111	121	79	80	101	74	110
BAX	95	78	100	91	110	106	109	100	91	91	92
DCK	112	80	176	164	91	101	132	102	99	91	117
HIF1B	129	106	56	99	65	112	65	102	566	76	107
EGFR	101	70	54	124	74	87	71	151	91	50	85
CCND1	128	106	171	127	85	107	141	127	107	69	97
GLI2	82	73	11	72	85	100	118	73	531	30	99
MAPK3	71	109	108	152	75	100	95	167	90	73	106
PLK1	34	36	15	50	31	30	21	98	90	68	111
NOTCH1	138	132	134	158	60	190	89	104	118	70	96
IGF1R	105	66	41	51	82	105	61	28	101	66	53
PSCA	127	115	81	106	132	110	102	91	101	108	94
VEGF	101	86	103	106	91	87	107	115	105	97	87
PTCH	102	53	93	81	153	97	48	63	203	33	78
CXCR4	5	43	13	100	0	70	229	5	342	53	649
ERCC1	78	88	94	107	150	106	105	153	98	91	91
IGFBP3	205	49	130	135	34	86	122	78	83	96	42
MAPK1	104	111	68	114	90	108	86	93	78	79	99
MAP2K2	78	106	146	127	98	135	151	131	86	89	90
JUN	109	114	83	118	106	95	135	80	108	82	92
MDM2	92	137	43	134	81	83	73	180	65	86	92
IHH	131	130	92	113	122	114	141	81	137	93	99

NOTE: Sensitivity to gemcitabine is shown above the case number and expressed as T/C ratio, where a negative value indicates tumor regression. Gene values indicate expression in percentage normalized to untreated control. Significant upregulation (red) and downregulation (green) were defined as a 2-fold increase and decrease in expression, respectively.

Statistical Analysis

The comparisons between means and proportions and the correlations obtained from the biological studies were done using Student's t test, χ^2 method, and Spearman's test, respectively, using $P = 0.01$ as the cutoff for significance to account for the multiple comparisons. Multiple comparisons were corrected by the Bonferroni's method.

Results

Xenograft Case Selection Based on Gemcitabine Sensitivity

We have tested gemcitabine in a panel of pancreatic cancer direct xenografts (3) and selected 11 cases with varying degrees of gemcitabine sensitivity ranging from

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almost complete tumor regression to absolutely refractoriness (Fig. 1A). With the aim of better understand the effect of gemcitabine in pancreatic cancer and to investigate which genes are related to gemcitabine responsiveness, we performed *ex vivo* testing where samples from untreated tumors acquired by FNA are seeded in a plate and exposed to control or 1 μmol/L gemcitabine for 6 h (Fig. 1B).

Ex vivo Assay Results: Plk1 Is the Common Denominator of Gemcitabine Resistance

Next, both samples were harvested for RNA, and the cDNA was run in a customized low-density array (Table 1). This low-density array contained 45 genes that had been selected based on their relevance to pancreatic cancer, their potential druggability, and being functionally regulated at the mRNA level. The gene expression of any of these genes in the untreated samples (which is considered a baseline, static readout) was not predictive of gemcitabine efficacy (data not shown). We considered a 2-fold variation to be of significance following standard gene expression methodology. Most (94%) of the 495 data cells remained stable, and for each tumor, a range of only one to five genes had significant variations in their expression after gemcitabine treatment.

Altogether, the only gene that differentiated sensitive versus resistant cases was Plk1 (Spearman's correlation, $P < 0.01$; rest of correlations, $P > 0.01$). In all the sensitive cases, Plk1 showed downregulation of ≥50%, whereas it did not change in any of the intermediate or resistant tumors (Fig. 2A). To determine the biological significance of changes in Plk1 expression in the low-density array, we correlated the expression of Plk1 after *ex vivo* exposure to gemcitabine and 28-day *in vivo* treatment in xenografts. As shown in Fig. 2B, there was a significant correlation between these two sample sets, suggesting that the *ex vivo* results predict the effect of a long-term *in vivo* exposure.

This experiment yielded three important outcomes. (*a*) It showed the feasibility of interrogating a relatively large group of genes of interest quantitatively with the small amount of material obtained from a FNA. (*b*) It evidenced that the majority of genes were not affected and that it is possible to obtain a focused readout. (*c*) It not only identified a marker predicting gemcitabine resistance (Plk1), but also a 6 h *ex vivo* exposure reproduced the events occurring in the tumors after a 28-day *in vivo* exposure to gemcitabine.

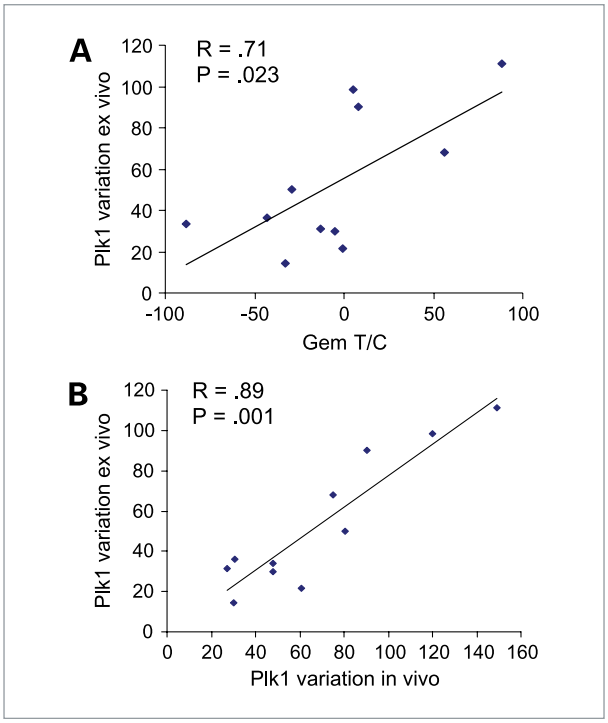

Figure 2. A, correlation between Plk1 variation *ex vivo* and gemcitabine sensitivity *in vivo*, expressed in T/C. B, correlation between Plk1 variation *ex vivo* and *in vivo*.

Plk1 Knockdown and Pharmacologic Inhibition Reverses Gemcitabine Resistance

To determine whether the decrease of Plk1 was a reflection of arrested growth or whether it was mechanistically involved in gemcitabine response, we knocked down Plk1 in a panel of six gemcitabine-resistant (defined as $IC_{50} > 1$ μmol/L) pancreatic cancer cell lines. The baseline levels of Plk1 did not correlate with gemcitabine sensitivity, and gemcitabine exposure did not decrease Plk1 expression in these cell lines. Plk1 knockdown alone had a modest growth-inhibitory effect on two of the cell lines (Fig. 3A), but the combination with gemcitabine induced a significant additive effect or synergy in three cell lines (1005, XPa3, and XPa4). Then, we treated the same panel of cell lines with gemcitabine ± the Plk1 pathway modulator ON 01910.Na (Fig. 3B). Similarly, whereas the effect of single-agent ON 01910.Na was marginal, the combination with gemcitabine induced synergy in three cell lines that were the same as those from the prior experiment. This validates the principle mechanistically and reinforces the notion that these cell lines seem to be sensitive to an interference with Plk1 function independently of the modality used.

Treatment of Three Xenograft Cases with Gemcitabine, a Plk1 Inhibitor, and the Combination

We then planned to validate these findings by treating *in vivo* three xenografts (410 intermediately sensitive to gemcitabine and JH033 and 286 resistant to gemcitabine) with gemcitabine, ON 01910.Na, and the combination of both (Fig. 4A). In 410, ON 01910.Na had a similar effect than gemcitabine and no additive effect was seen with the combination. In JH033, the combination showed a marginally better effect than either modality alone. In 286, however, a case where gemcitabine had no effect and ON 01910.Na only modestly slowed growth, the combination induced tumor regressions. This suggests that the higher gemcitabine resistance was *in vivo*, the more benefit was obtained from a combination with a Plk1 inhibitor.

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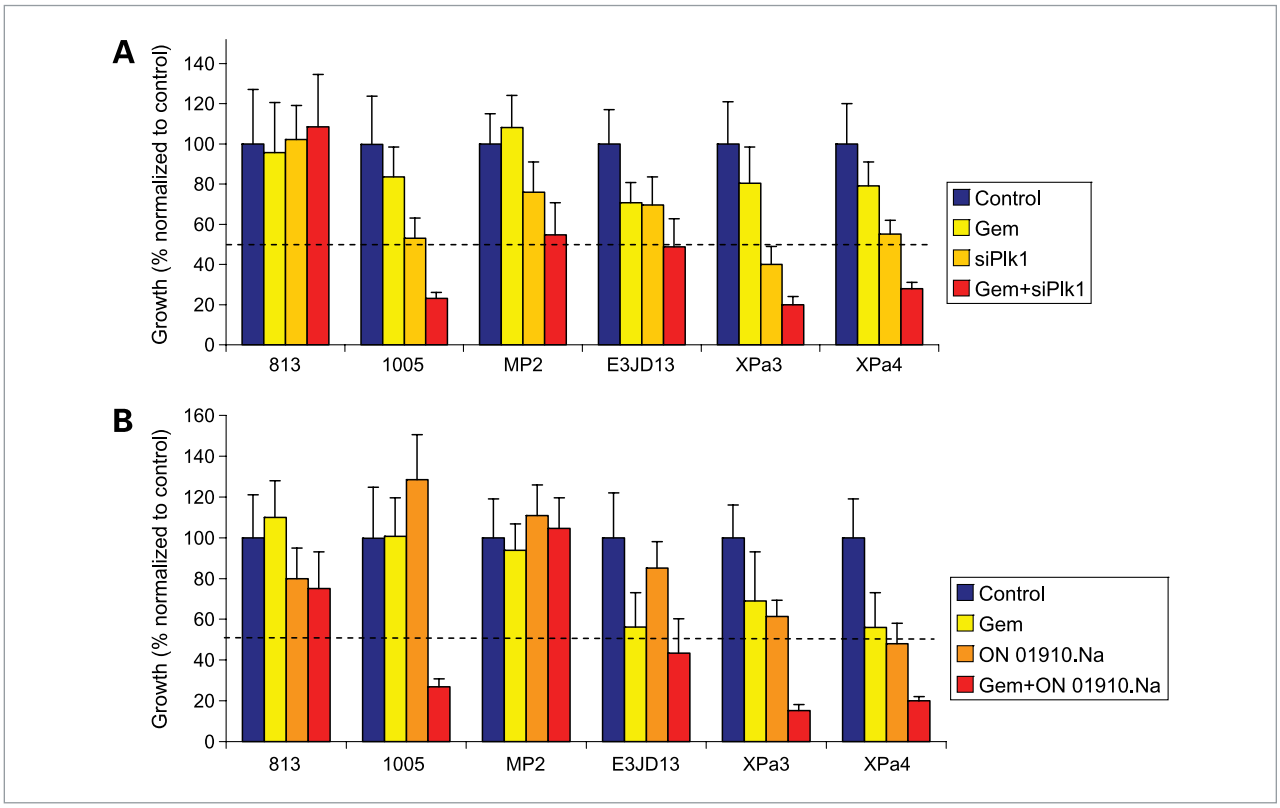

Figure 3. A, a panel of six gemcitabine-refractory pancreatic cell lines. Gemcitabine ± Plk1 knockdown induced a significant additive effect or synergy in three cell lines (1005, XPa3, and XPa4). B, the same panel was treated with gemcitabine ± the Plk1 inhibitor ON 01910.Na. Whereas the effect of single-agent ON 01910.Na was marginal, the combination with gemcitabine induced synergy in the same three cell lines.

In the analysis of the tumors, Plk1 mRNA levels did not significantly change in any of the arms of any of the three cases (Fig. 4B). Cyclin B1, a transducer distal to CDC25C, significantly reduced its nuclear levels in both the ON 01910.Na and the combination arms of JH033 (Fig. 4C).

Discussion

In this work, we aimed at determining the factor(s) that dictates the vulnerability of pancreatic cancer to gemcitabine using a direct xenograft platform and a dynamic, FNA-based *ex vivo* drug exposure approach with a gene expression endpoint on a customized, multigene, quantitative array. We used 11 cases with known gemcitabine sensitivity and found that the common feature of resistant cases was an inability of gemcitabine to induce a downregulation of Plk1. This could have two interpretations: it could represent a proliferation-related event, whereby only in cases where gemcitabine is effective a nonspecific decrement in cycling parameters occurs, or it could indicate a bona fide mechanistic event. The series of experiments that followed suggest that the latter is more likely: downregulation of Plk1 by using siRNA-induced antitumor efficacy per se but more importantly synergized with gemcitabine in gemcitabine-refractory models; additionally, a parallel pharmacologic inhibition with an agent that targets the Plk1 pathway exerted a similar effect both in terms of proliferation but also pharmacodynamically in the exact same subgroup of cells. This effect, however, was not universal, and the determinants dictating synergy in *in vitro* models are unknown and under investigation at the time of this report. Then, this was validated *in vivo*, and only the combination of gemcitabine with ON 01910.Na, a Plk1 pathway inhibitor, induced tumor regressions. It should be noted that multiple combinations of agents (including several gemcitabine-based agents) have been tested in 286 without success.

Plk1 is one of the key mediators of the intricate and overlapping control points into the mitotic phase (9, 10) and modulates the transition through the G_2-M checkpoint in the cell cycle by influencing the activation of the phosphatase CDC25C and cyclin B1 (11). To examine the role of Plk1, several approaches have been explored. Deletion mutants of Plk1 delayed progression through mitosis, and when Plk1 function was blocked through adenovirus delivery of a dominant-negative gene, tumor-selective apoptosis in tumor cell lines was observed (12). Downregulation of cellular Plk1 levels in cancer cells by using antisense oligonucleotides altered cell cycle progression and resulted in antiproliferative

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effects in *in vitro* and *in vivo* models of lung cancer (13) as well as in pancreatic cancer cell lines (14). Short hairpin RNAs against Plk1 reduced tumor growth in cervical and lung cancer xenograft models (15). siRNA-mediated Plk1 depletion inhibited cell proliferation, decreased viability, resulted in cell cycle arrest with 4N DNA content, and induced apoptosis; the latter effect was partially reversed by cotransfection of nondegradable mouse Plk1 constructs (16). This has validated Plk1 as an anticancer target (17), but these approaches are difficult to translate to the clinic. The relevance of the Plk1 pathway in pancreatic cancer was highlighted by expression analyses showing that Plk1 was differentially overexpressed in pancreatic cancers as early as pancreatic intraepithelial neoplasia III lesions as opposed to benign acinar pancreatic parenchyma and ductal epithelia, where only focal Plk1 positivity was evidenced (18). A separate analysis found Plk1 mRNA and protein to be overexpressed in clinical pancreatic cancer samples and in cell lines but more importantly decreasing Plk1-induced growth arrest in preclinical models (14). Yu et al. showed recently that treatment of pancreatic cancer cells with Plk1 siRNA followed by exposure to gemcitabine dramatically decreased cell viability and increased cellular apoptosis compared with treatment with either agent alone (19).

Elegant mechanistic studies provide the biological framework that explains the results observed. In undamaged cells, several redundant pathways can promote the onset of mitosis; this redundancy is lost in cells recovering from a DNA damage–induced arrest (20). Plk1 is crucial for mitotic entry following recovery from DNA damage; therefore, our results would fit the hypothesis that cells with functional overexpression of Plk1 that does not decrease after gemcitabine-induced insult are ultimately resilient to arrest and senescence/death. This explains why only a dual/sequential targeting with gemcitabine and a Plk1 inhibitor results in cell death and tumor growth arrest. It also explains that in cases with intermediate or full susceptibility to gemcitabine Plk1 inhibition fails to increase this effect, as it is likely that susceptibility arises from the tumor cell undergoing apoptosis after entering an ineffective cell division. These are the cases with high Plk1 function where cell division after DNA damage does not occur, which derives benefit from Plk1 inhibition.

This work highlights the potential value of a FNA-based *ex vivo* assay for pharmacodynamic assessment of cancer (4). If validated clinically, this approach may have implications in drug development, as it (*a*) permits identifying which molecules or mediators are particularly relevant in determining the vulnerability to a specific drug and is a rational way to devise combinatorial strategies and eventually (*b*) will allow identifying which patients may obtain benefit before receiving the drug, taking a step forward in the implementation of

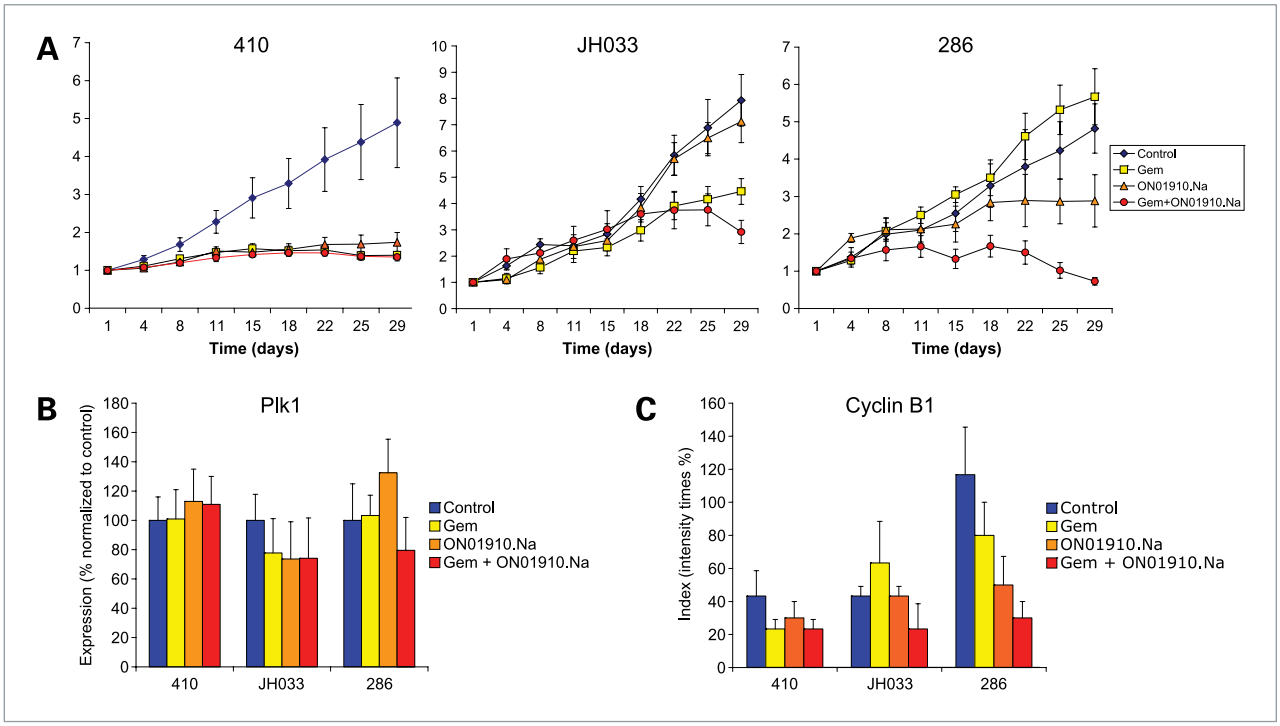

Figure 4. A, tumor growth plots of the *in vivo* experiments. Tumor-bearing mice were distributed in four groups that were treated with vehicle, gemcitabine, ON 01910.Na, and the combination of both agents (at full doses). B, 12 tumors per case (3 per treatment arm) were assessed by reverse transcription-PCR. Plk1 levels did not significantly change in any of the arms of any of the three cases. C, 3 tumors per treatment group were examined by immunohistochemistry for cyclin B1 expression. In 286 in the combined arm, there was a significant downregulation of cyclin B1. Bars, SD.
*, $P < 0.05$, compared with control (Student's *t* test).

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individualized therapy. FNA-based acquisition of tumor material, particularly from nodal or hepatic origin is feasible, safe, and effective as is usually coupled to ultrasound guidance, and the quality of the procedure is tested on-site by cytopathologic assessment. This is in contrast to thoroughly tested sensitivity and resistance assays that require a larger amount of material (5, 6), typically limiting their use to patients that can undergo a surgical resection or for superficial tumors (21). A key to successfully develop a clinical assay is choosing an endpoint that can be tested in a reasonably large proportion of the potential patients. mRNA expression analysis by reverse transcription-PCR requires less material, is reproducible and fully quantitative, and thus has a higher potential for miniaturization. This effort is complementary and has similar intent as high-throughput lethality assays using gene interference to knockdown target genes in combination with conventional chemotherapy as a strategy to rationally identify combination partners (22). The ability to knockdown genes requires an *in vitro* platform such as a panel of cell lines.

Recent work has shown the enhancement of gemcitabine sensitivity with concomitant use of hedgehog inhibitors that maximize pharmacokinetic gemcitabine delivery by modulating the stromal components (23). Here, we present a molecular mechanism making gemcitabine more effective on an equimolar basis. These mechanisms do not appear to be mutually exclusive, and it would be fascinating to determine in future work whether both gemcitabine-enhancement methods can be used in concert.

In summary, a quantitative gene expression–based vulnerability assay has identified Plk1 as relevant in dictating the susceptibility of pancreatic cancer to gemcitabine. Dynamic interrogation of cancer has the potential to provide key information about mechanisms of resistance and to enhance our capability to individualize therapy.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References

1. Jemal A, Siegel R, Ward E, Murray T, Xu J, Thun MJ. Cancer statistics, 2007. CA Cancer J Clin 2007;57:43–66.
2. Yeo TP, Hruban RH, Leach SD, et al. Pancreatic cancer. Curr Probl Cancer 2002;26:176–275.
3. Rubio-Viqueira B, Jimeno A, Cusatis G, et al. An *in vivo* platform for translational drug development in pancreatic cancer. Clin Cancer Res 2006;12:4652–61.
4. Hidalgo M, Amador ML, Jimeno A, et al. Assessment of gefitinib- and CI-1040-mediated changes in epidermal growth factor receptor signaling in HuCCT-1 human cholangiocarcinoma by serial fine needle aspiration. Mol Cancer Ther 2006;5:1895–903.
5. Samson DJ, Seidenfeld J, Ziegler K, Aronson N. Chemotherapy sensitivity and resistance assays: a systematic review. J Clin Oncol 2004;22:3618–30.
6. Schrag D, Garewal HS, Burstein HJ, Samson DJ, Von Hoff DD, Somerfield MR. American Society of Clinical Oncology Technology Assessment: chemotherapy sensitivity and resistance assays. J Clin Oncol 2004;22:3631–8.
7. Jimeno A, Kulesza P, Kincaid E, et al. c-fos assessment as a marker of anti-epidermal growth factor receptor effect. Cancer Res 2006;66:2385–90.
8. Jones S, Zhang X, Parsons DW, et al. Core signaling pathways in human pancreatic cancers revealed by global genomic analyses. Science 2008;321:1801–6.
9. Nigg EA. Polo-like kinases: positive regulators of cell division from start to finish. Curr Opin Cell Biol 1998;10:776–83.
10. Smits VA, Klompmaker R, Arnaud L, Rijksen G, Nigg EA, Medema RH. Polo-like kinase-1 is a target of the DNA damage checkpoint. Nat Cell Biol 2000;2:672–6.
11. Jackman M, Lindon C, Nigg EA, Pines J. Active cyclin B1-1 first appears on centrosomes in prophase. Nat Cell Biol 2003;5:143–8.
12. Mundt KE, Golsteyn RM, Lane HA, Nigg EA. On the regulation and function of human polo-like kinase 1 (PLK1): effects of overexpression on cell cycle progression. Biochem Biophys Res Commun 1997;239:377–85.
13. Spankuch-Schmitt B, Wolf G, Solbach C, et al. Downregulation of human polo-like kinase activity by antisense oligonucleotides induces growth inhibition in cancer cells. Oncogene 2002;21:3162–71.
14. Gray PJ, Jr., Bearss DJ, Han H, et al. Identification of human polo-like kinase 1 as a potential therapeutic target in pancreatic cancer. Mol Cancer Ther 2004;3:641–6.
15. Spankuch B, Matthess Y, Knecht R, Zimmer B, Kaufmann M, Strebhardt K. Cancer inhibition in nude mice after systemic application of U6 promoter-driven short hairpin RNAs against PLK1. J Natl Cancer Inst 2004;96:862–72.
16. Liu X, Erikson RL. Polo-like kinase (Plk) 1 depletion induces apoptosis in cancer cells. Proc Natl Acad Sci U S A 2003;100:5789–94.
17. Barr FA, Sillje HH, Nigg EA. Polo-like kinases and the orchestration of cell division. Nat Rev Mol Cell Biol 2004;5:429–40.
18. Weichert W, Schmidt M, Jacob J, et al. Overexpression of polo-like kinase 1 is a common and early event in pancreatic cancer. Pancreatology 2005;5:259–65.
19. Yu C, Zhang X, Sun G, et al. RNA interference-mediated silencing of the polo-like kinase 1 gene enhances chemosensitivity to gemcitabine in pancreatic adenocarcinoma cells. J Cell Mol Med 2008;12:2334–49.
20. van Vugt MA, Bras A, Medema RH. Polo-like kinase-1 controls recovery from a G_2 DNA damage-induced arrest in mammalian cells. Mol Cell 2004;15:799–811.
21. Ugurel S, Schadendorf D, Pfohler C, et al. *In vitro* drug sensitivity predicts response and survival after individualized sensitivity-directed chemotherapy in metastatic melanoma: a multicenter phase II trial of the Dermatologic Cooperative Oncology Group. Clin Cancer Res 2006;12:5454–63.
22. Azorsa DO, Gonzales IM, Basu GD, et al. Synthetic lethal RNAi screening identifies sensitizing targets for gemcitabine therapy in pancreatic cancer. J Transl Med 2009;7:43.
23. Olive KP, Jacobetz MA, Davidson CJ, et al. Inhibition of Hedgehog signaling enhances delivery of chemotherapy in a mouse model of pancreatic cancer. Science 2009;324:1457–61.

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