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14. ABSTRACT

Pancreatic cancer is a highly lethal malignancy with an expected 5-year survival of less than 5% for all patients using current therapies. Most of these therapies rely on inducing DNA damage and replication blocks to cause cell death; however the effectiveness of these treatments is often limited to a subset of patients that respond to treatment. Understanding why patients are sensitive or resistant to specific treatments would allow the personalization of therapies that are most effective for a patient while potentially reducing toxicity. The Replication Stress Response (RSR) is a signaling network that recognizes challenges to DNA replication and mobilizes diverse activities to maintain genome integrity. The RSR is critical for the prevention of pancreatic cancer by acting as a barrier against genomic instability and tumorigenesis. We hypothesized that novel RSR genes maintain genome integrity by participating in an ATR-mediated replication stress response and that dysregulation of RSR genes and their cancer barrier function results in the development of pancreatic cancer. Utilizing a custom generated siRNA library targeting genes somatically mutated in pancreatic cancer from the Sanger COSMIC library, we completed a synthetic lethal screen to identify genes which when silenced mediate gemcitabine sensitivity in human pancreatic cancer cells. We further validated positive hits be deconvolution of individual siRNAs and began work on determining their activities in DNA replication and DNA damage responses.

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Introduction

Pancreatic cancer is a highly lethal malignancy with an expected 5-year survival of less than 5% for all patients using current therapies. Most of these therapies rely on inducing DNA damage and interfering with DNA replication to cause cell death; however the effectiveness of these treatments is often limited to a subset of patients that respond to treatment. Understanding why patients respond or do not respond to specific treatments would allow the personalization of therapies that are most effective for a patient while potentially reducing toxic side effects. The Replication Stress Response (RSR) is a signaling pathway that recognizes challenges to DNA replication and mobilizes diverse activities to maintain genome integrity. The RSR is critical to prevent pancreatic cancer. In human pre-cancerous lesions, aberrant DNA replication induces activation of the RSR, which maintains genome integrity or causes cell death. Mutations in the RSR promote the survival and proliferation of genetically unstable cells ultimately resulting in cancer. However, the genetic changes that lead to pancreatic cancer can also weaken the ability of cancer cells to respond to treatment by compromising DNA repair pathways. Often the cancer cell will become reliant on backup pathways, which can be targeted to cause cell death through the principle of synthetic lethality. Two genes or pathways are synthetically lethal when inactivation of one is sublethal but inactivation of both causes cell death. We hypothesize that novel RSR genes maintain genome integrity and that deregulation of RSR genes and their cancer barrier function results in the development of pancreatic cancer. However, the genetic changes that drive pancreatic cancer progression also cause susceptibility to synthetic lethal cancer treatments, which can be exploited to personalize therapy. To test this hypothesis, we propose the following specific aims: 1) Identify RSR genes which mediate sensitivity to the first-line chemotherapeutic agent gemcitabine in pancreatic cancer cells. 2) Determine the activities of RSR proteins in DNA replication and DNA damage responses. 3) Evaluate if RSR genes can function as biomarkers for response in pancreatic cancer. Completion of these aims will provide new insights into how the RSR maintains genome integrity, elucidate novel targets for the treatment of pancreatic cancer, and identify subsets of pancreatic cancers that may benefit from gemcitabine chemotherapy.

Body

We completed a loss of function genetic screen to identify genes, which when silenced cause sensitization or resistance to a low dose of gemcitabine, in human pancreatic cancer cells. We reasoned that genes involved in the RSR would likely be involved in the ATR signaling pathway. We therefore optimized a high-throughput assay using ATR or CHK1 siRNA oligonucleotides as positive controls and ATM or non-targeting (NT) oligonucleotides as negative controls with cell proliferation as a read-out (Fig. 1A-B). The primary screen was completed in MIA PaCa-2 cells, which consistently gave the highest signal to noise ratio among several tested cell types (data not shown).

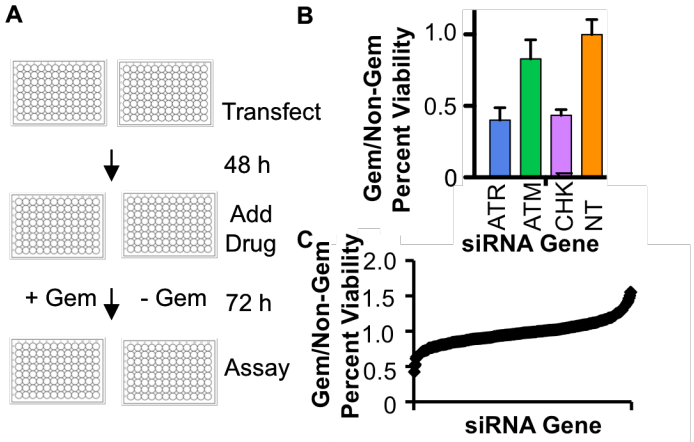

Fig. 1. Gemcitabine sensitivity screen. A. Primary screen flow diagram. MIA PaCa-2 cells were transfected with siRNA (pools of 4 siRNAs per gene) in a one gene/one well format in 96-well plates. 48 hours after transfection, cells were treated with or without 13 nM gemcitabine for 72 hours prior to assaying for cell proliferation using WST-1 reagent. In each plate, non-targeting (NT) targeting, ATR, and CHK1 siRNA were used as controls. Plate-to-plate variability was controlled by normalizing the values on that plate to the average of the negative controls on that plate. **B.** Optimization of cell viability assay using ATR, ATM, CHK1, or NT siRNA. The viability ratio of gemcitabine treated to untreated is shown. Mean and standard deviation from three replicas is shown. **C.** Results of primary screen. The primary screen was completed in triplicate. The viability ratio of treated versus untreated cells is shown.

Briefly, cells were reverse transfected with pools of 4 siRNAs targeting a unique sequence of each gene arrayed in a one gene/one well format in 96-well plates. Forty-eight hours after transfection, cells were treated with or without 13 nM gemcitabine for 72 hours prior to assaying for cell proliferation using WST-1 reagent. Each plate contained two positive controls (ATR and CHK1) and several negative controls (NT), and plate-to-plate variability was controlled by normalizing the values on each plate to the average of the negative control values on that plate. We completed three replicas of the primary screen using a library of 1540 siRNAs, corresponding to four unique siRNA duplexes, targeting each of 385 unique human genes (Fig. 1C) somatically mutated in pancreatic cancer from the Sanger COSMIC database[1] (Task 1-1). Positive hits included a number of genes previously linked to DNA replication and/or DNA damage responses, including *FANCI*, *BRCA2*, *PKP2*, and *CTBP2*, demonstrating that our screen can yield genes involved in the RSR. We performed bioinformatic analyses of our positive hits including cross-referencing with published putative ATM/ATR substrates[2] and published DNA damage sensitivity screens and putative ATM/ATR substrates[3-6] (Task 3-1). Using these criteria, we selected 20 genes for further analysis in a secondary screen by deconvoluting with individual siRNAs (Task 1-2). Analysis of this data is pending. We further optimized conditions for analyzing gemcitabine sensitivity of PANC-1 and BxPC3 cells silenced for novel gemcitabine sensitivity genes (Task 1-3). We further analyzed the gemcitabine sensitivity of pancreatic cell lines ASPC1, MIA PaCa-2, PANC1, HPAC, BxPC3, Capan-1 and, Capan-2 cells and found that PANC1 cells are significantly more resistant to gemcitabine than the other cell lines (Task 3-6).

Key Research Accomplishments

1. Optimized conditions for novel gemcitabine sensitivity genes in human pancreatic cancer cells.

2. Completed primary gemcitabine sensitivity screen.

3. Completed bioinformatic analyses of positive hits.

4. Completed secondary validation screen of positive hits by deconvoluting with individual siRNAs.

5. Determined gemcitabine sensitivity of panel of pancreatic cancer cell lines.

Reportable Outcomes

Seminars:
"Pancreatic Cancer Research and Challenges," Seminar, Winship Cancer Institute Pancreatic Cancer Support Group, January, 2013.

"Understanding and Exploiting the Replication Stress Response in Cancer," Seminar, Division of Cancer Biology Seminary Series, Emory University School of Medicine, February, 2013.

National Meetings:
"Rationale-Driven Identification of Novel Gemcitabine Sensitivity Genes as Biomarkers for Outcome in Pancreatic Cancer Patients by Exploiting the Replication Stress Response," 3rd World Congress on Cancer Science and Therapy, San Francisco, CA, October, 2013.

Conclusion

We hypothesized that novel RSR genes maintain genome integrity and that dysregulation of RSR genes and their cancer barrier function results in the development of pancreatic cancer. However, the genetic changes that drive pancreatic cancer progression also

cause susceptibility to synthetic lethal cancer treatments, which can be exploited to personalize therapy. To test this hypothesis, we completed a loss of function genetic screen using a siRNA library of genes somatically mutated in pancreatic cancer to identify genes which when silenced cause sensitivity or resistance to a low dose of gemcitabine treatment. These genes many function as potential biomarkers for outcome to gemcitabine therapy or novel targets to be used as an adjunct to gemcitabine treatment to personalize pancreatic cancer therapy

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Appendices

N/A

Supporting Data

See above.