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ORIGINAL RESEARCH

Molecular Alterations Associated with DNA Repair in Pancreatic Adenocarcinoma Are Associated with Sites of Recurrence

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Abstract

Background Pancreatic ductal adenocarcinoma (PDAC) remains one of the deadliest malignancies with a rising incidence. Mutational analysis of PDAC has provided valuable information but has not yet dramatically changed the therapeutic landscape due to the number of variations detected in any one individual. The pattern of molecular alterations—gene mutations, variations in copy number, and changes in gene expression—has been described in the literature. The purpose of this study is to further investigate the molecular alterations in recurrent or metastatic PDAC based on the site of disease.

Methods Molecular alterations in patients with recurrent or metastatic PDAC from 2007 to 2015 were analyzed. The most common molecular alterations found in PDAC tumors from the pancreas were compared to metastatic PDAC specimens from the liver, lung, peritoneum, and other locations. Means were compared with a two-tailed Student's *t* test or ANOVA as appropriate. Rates of molecular alterations among the different groups were compared with Pearson's χ^2.

Results Two thousand five hundred fifty-two patients with PDAC were identified in a retrospective database, and the 15 most common molecular alterations were utilized for analysis. The most common alterations among all patients were mutations in *KRAS* and *PTEN* (59 and 62%, respectively), with differences in prevalence by site of metastasis ($p = 0.042$ and $p = 0.037$, respectively). *KRAS* mutations were more commonly found in metastasis in the lung (72%) than in other sites (59%, $p = 0.042$). Low expression of *ERCC1* was found in 49% of lung metastases from PDAC but only 15% in PDAC in the pancreas ($p < 0.001$). Five of the 8 molecular alterations significantly associated with site of metastatic disease were involved in DNA maintenance, repair, replication, or transcription (each $p < 0.001$).

Conclusions Aberrant expression or mutation in genes involved in DNA maintenance is found in association with specific sites of metastatic PDAC. Personalizing therapy for metastatic PDAC based on site of disease and their associated molecular alterations warrants further investigation.

Keywords Pancreatic adenocarcinoma · Genetic mutations · Gene expression profiling · Molecular alterations · Genomic variations

Introduction

Pancreatic ductal adenocarcinoma (PDAC) is the third leading cause of cancer-related death and an estimated 53,070 new cases of pancreatic cancer were diagnosed in 2016 [1]. The estimated number of deaths from PDAC in 2016 was reported as 41,780, totaling 7% of all cancer-related deaths. Investigations of broad genomic analysis and more targeted molecular alterations in PDAC have yielded interesting results, but have not significantly changed therapeutic approaches [2].

Others have demonstrated that the site of PDAC metastasis is related to survival [3]. For instance, pulmonary metastases may follow a more indolent course relative to local recurrence or metastases to other sites [4]. Though most PDAC will relapse with local or metastatic disease, the pattern of molecular alterations for these sites of recurrence has not been well described. While molecular alterations are established in PDAC,

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targeted therapy has not been as successful in PDAC as other cancers [5]. Importantly, there are variations in response to standard therapy based on the four subtypes of PDAC but the standard treatments remain the same [5, 6]. There is "biological priming" in certain metastatic sites that encourages the progression of PDAC metastases [7] based on the "soil" model of distant disease [8]. Better delineation of individual tumor genomic heterogeneity for any given patient with PDAC represents an opportunity to develop personalized therapy [9]. This might represent an opportunity to "reprogram" the genome and epigenome during tumor progression which seems to direct tumor-specific activities such as metastases and glucose metabolism [10].

Routine profiling of molecular alterations has been an integral component of our recent clinical practice for patients with recurrent or metastatic PDAC. Our group has published investigations of uncommon tumor types in order to better understand tumor biology and guide therapy [11–13]. Similar to many centers, translational applicability has been tempered by limited therapeutic options and the difficulty in ascertaining difference between driver and passenger alterations [14]. However, PDAC, a relatively common malignancy with a high fatality rate, is a disease process likely to benefit from a personalized molecular therapy approach if (1) certain genomic aberrations can be identified and (2) current available therapies can be therapeutically utilized toward these unique situations.

The purpose of our study is to delineate the molecular alterations in advanced PDAC based on the site of metastatic disease. We hypothesize that the site of metastasis from PDAC will be associated with a specific pattern of molecular alterations. Previously, using in silico analysis has helped identify potential biomarkers and therapeutic options that would not have been routinely identified [15].

Materials, Methods, and Patients

Patients

Caris Life Sciences (Dallas, TX), a biotechnology company with a CLIA and College of American Pathology approved laboratory, profiled PDAC tumors to investigate molecular alterations (standard of care testing). Patients with PDAC were profiled from 2007 to 2015. Specimens were analyzed from multiple centers across the USA, and all specimens are formalin fixed, paraffin embedded. All patients have recurrent and/or metastatic disease. This study was performed using tissue specimens of metastatic and recurrent PDAC tumors. Data were de-identified from Caris Life Sciences. Limited characteristics of the patients included age, gender, and specimen site. The West Cancer Center Institutional Review Board approved this study.

The inclusion criteria were all patients with a histologic diagnosis of pancreatic ductal adenocarcinoma who had molecular alteration testing with a known site of biopsy. Excluded patients were those who had incomplete data or specimens analyzed before 2007 or after 2015. Only the first site of recurrence or metastases was utilized.

The sites of disease included pancreas (pancreas, peripancreatic tissue), liver (liver and extrapancreatic bile ducts), peritoneum (peritoneal lining, intraperitoneal space, retroperitoneum, omentum, surface of gastrointestinal organs, mesentery), lung (lung, hilum, mediastinal lymph nodes), and other sites (brain, skin, distant lymph nodes, spleen, duodenum, stomach, adnexa, vertebra, ovary, diaphragm). The patients were divided into groups for analysis based on the site of recurrent or metastatic PDAC disease.

Genes, Proteins, and Molecular Alterations

The molecular testing includes protein expression analysis (immunohistochemistry [IHC]), gene sequencing (Sanger or next-generation sequencing [NGS]), and gene copy number variation (CNV) and translocation analysis (chromogenic or fluorescence in situ hybridization [CISH or FISH, respectively]). Up to 623 molecular alterations—gene mutations, changes in IHC expression, and CNV—were analyzed for each specimen. The 15 most common molecular alterations in the entire study population were further analyzed in detail for this study.

The list of genes and proteins investigated has been well described previously by our group and others [11–13, 15]. De-identified data and limited patient characteristics were obtained from a retrospective database of all patients with PDAC. Analysis of specific molecular alteration is defined as a "test." An abnormal test result is defined as "positive" while all other results (normal, negative, or incomplete) and variants of unknown significance were considered "negative" for the purpose of this study. If a specific test was not performed for a specific specimen, then it was excluded from the denominator and excluded for that test. The top 15 most common positive tests were determined based on the proportion of positive results for each test.

Statistical Approach and Statistical Analyses

Disease site group comparisons were performed for the most common molecular alterations found in tumors from the pancreas and compared to metastatic lesions in the liver, lung, peritoneum, and other locations. Statistical analysis was conducted using STATA/IC 14 (StataCorp LP, College Station, TX). The mean age for two group comparisons were compared with Student's t test while analysis of variance (ANOVA) was used to compare the mean age of patients for three or more groups. Rates of molecular alterations among

the different groups were compared with Pearson's χ^2. Standard deviations of the mean are provided where appropriate. A modified Bonferroni correction was used to adjust the threshold for statistical significance due to multiple comparisons. Hence, $\alpha < 0.003$ was used for statistical significance by dividing the standard $p = 0.05$ by the number of potential parameters, 15 (i.e., 0.003 = 0.05/15).

Results

Molecular Alterations Vary Based on the Site of PDAC Recurrence

A total of 2552 subjects met the inclusion criteria for this study. Overall, 54% of the subjects were male with a mean age of 63 ± 10 years (Table 1). Differences in the mean age among the groups was not statistically significant ($p = 0.63$), whereas subjects with metastasis to the liver were more likely to be male compared to subjects with lung metastases ($p = 0.002$). Recurrent PDAC tumors represented the largest proportion of specimens examined and the liver was the most frequent site of metastasis. Patients with lung metastases were significantly less likely to be male ($p = 0.002$). Overall, 510,113 tests were performed on 2552 individuals with 5.6% of all tests being positive. The 15 most common molecular alterations were utilized for analysis (Table 2). Eight of these tests reached statistical significance based on the corrected p value for multiple comparisons (Table 2; each $p < 0.003$). Variability, as shown in Table 2, denotes the standard deviation, a measure of the difference between the lowest and highest levels in each group.

The most common molecular alterations for all patients in the study were mutations in *KRAS* and *PTEN* (59 and 62%, respectively), and both demonstrated slight differences in the prevalence among the different sites of metastases (Table 2; both $p = 0.04$). Low expression of *ERCC1*, a protein in the nucleotide excision repair pathway, was seen in 49% of lung metastases from PDAC but only in 15% of locally recurrent PDAC in the pancreas ($p < 0.001$). *KRAS* mutations were more commonly found in metastases to lung than in those to other sites (73 vs 59%, respectively; $p = 0.042$). There were no differences in *EGFR* expression or mutation among the various disease sites ($p = 0.5$).

Molecular Alterations Associated with the Disease Site Are Involved in DNA Maintenance

Five of the 8 molecular alterations associated with disease site are involved in DNA repair, replication, or transcription (*ERCC1*, *TOPO1*, *TOP2A*, *TS*, and *TLE3*; all $p < 0.001$). The remaining 3 alterations included *TUBB3*, *cMET*, and *PGP* (all $p < 0.001$). All of the statistically significant molecular alterations were increased expression based on IHC with the exception of *cMET* where some tumors demonstrated gene mutation based on next-generation sequencing.

The Site of Recurrence Is Associated with the Rate of Molecular Alterations

In our analysis, molecular variability of PDAC was mostly dependent on the site of disease. Of the 623 molecular tests for each specimen, the rate of positive results was the lowest for locally recurrent PDAC tissue obtained from the pancreas (4.9%) when compared to the other metastatic sites, especially the liver (6.6%, Fig. 1, $p < 0.001$). Overall, this results in approximately 30 to 40 molecular alterations per patient specimen. The second highest per-patient rate of molecular alterations was found in the lung (6.1%).

The molecular alteration rate of the 15 most common abnormalities vary by PDAC metastatic site (Fig. 2, $p < 0.001$). Some molecular alteration rates are similar throughout each metastatic site (i.e., expression of *MGMT*, $p = 0.09$, orange in Fig. 2). *TOPO1* has over a twofold difference in abnormal expression rates based on the site of disease ($p < 0.001$). Despite *cMET* overexpression occurring in a minority of any one disease site (Fig. 2, bright blue), statistically significant differences remain ($p < 0.001$).

Discussion

Our data demonstrates a diverse picture of metastatic PDAC and is similar to that of many other malignancies. The current treatment for metastatic or recurrent PDAC is to treat with an as aggressive and intensive chemotherapy regime as the patient will tolerate. Personalizing the treatment for specific site of metastatic disease is currently not standard of care. The results here suggest that molecular

Table 1 Patient characteristics based on site of recurrence or metastasis

	Pancreas	Liver	Peritoneal	Other	Lung	*p* value
Age, mean ± SD years	64 ± 10	62 ± 10	63 ± 11	63 ± 10	64 ± 10	0.63
Male, %	53%	58%	51%	50%	40%	0.002
Proportion of study, *n* (%)	1075 (42%)	882 (35%)	209 (8%)	260 (10%)	126 (5%)	–

p value represents the probability that age or gender is statistically different among the groups

Table 2 Distribution of molecular alterations in PDAC based on site of recurrence or metastasis

	Pancreas (%)	Liver (%)	Peritoneal (%)	Other (%)	Lung (%)	*p* value	Variability (%)
TOPO1	35	72	63	58	65	*< 0.001*	14
TOP2A	46	77	64	58	47	*< 0.001*	13
ERCC1	15	34	33	27	49	*< 0.001*	12
PGP	59	50	57	56	75	*< 0.001*	9
TUBB3	51	58	50	41	43	*< 0.001*	7
TS	21	23	15	27	10	*0.001*	7
PD-1	39	32	21	30	34	0.005	7
TLE3	34	48	33	34	35	*< 0.001*	6
KRAS	68	69	68	59	72	0.042	5
TP53	50	54	49	41	47	0.028	5
MGMT	70	75	75	77	81	0.094	4
MLH1	15	19	13	17	23	0.32	4
cMET	22	31	31	28	29	*< 0.001*	4
EGFR	23	25	26	21	27	0.533	2
PTEN	34	39	37	38	39	0.037	2

P values represent statistically significance among the five groups. Variability represents the average percent difference between the overall molecular alteration rate and the molecular alteration rate in each group for each gene

Statiscally signficant values ($P<0.05$) are italized

alterations in different sites of PDAC disease may have unique alterations. While it is too preliminary to suggest basing systemic chemotherapy on the PDAC tumor location, patients who partially respond in one metastatic site, but not others, may warrant additional consideration to identify genomic alterations at the non-responding site.

The diversity of metastatic and recurrent PDAC is demonstrated with the numerous molecular alterations described here. Variations in molecular alterations—gene mutations, changes in protein expression, and gene copy number variation—varied from close to zero to nearly 80% on a gene-by-gene basis. Most individual tumors had between 30 and 40 molecular alterations of the 623 molecular variants studied in this investigation. Our study again demonstrated that the most common site of extrapancreatic PDAC disease was the liver but other sites of metastatic spread disease were commonly found (Table 1).

The difference in the site of metastatic disease between males and females was an unexpected finding (Table 1). We found only a slight preponderance of subjects with metastatic PDAC to be male except for lung metastases where 60% of subjects were female. Further investigation regarding this disparity may help describe mechanism of metastatic development.

Investigations of molecular alterations in patients with PDAC will likely yield useful data to guide patient therapy

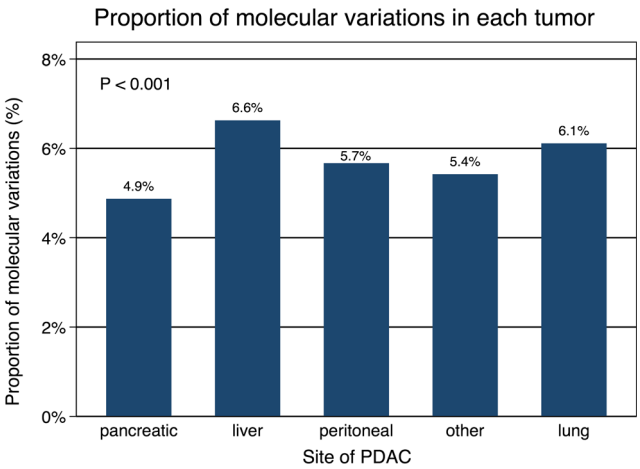

Fig. 1 Of the 623 molecular tests for each specimen, the rate of positive results was the lowest for locally recurrent PDAC tissue obtained from the pancreas (4.9%) when compared to the other metastatic sites, especially the liver (6.6%, Fig. 1, overall $p < 0.001$)

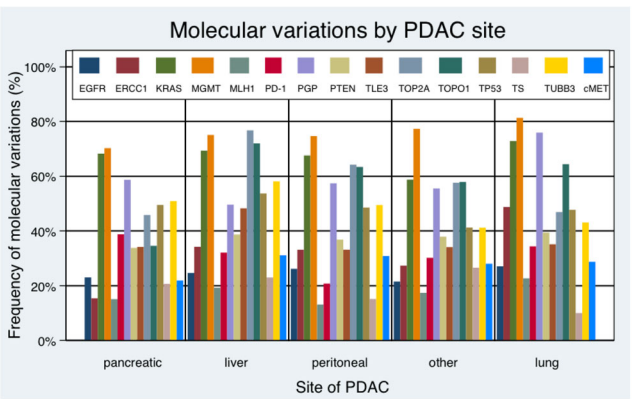

Fig. 2 The molecular alteration rate of the 15 most common abnormalities vary by PDAC metastatic site (Fig. 2, $p < 0.001$)

when targeted therapy is more effective. While the current staging system for PDAC groups all metastatic sites of disease together, the future of PDAC management may increasingly be defined by molecular subtyping [2, 9, 10, 16, 17]. For cholangiocarcinoma and appendiceal carcinoma, it has been suggested that the presence of *TOPO1* mutation favors irinotecan therapy since the cancer cell may have limited ability to develop resistance again this therapy [18, 19]. A similar approach of exploiting PDAC cells intrinsic genomic alterations may help personalize therapy in the future.

Interestingly, 5 of the 8 molecular alterations significantly associated with the site of disease serve roles in DNA repair, replication, and transcription (*ERCC1*, *TOPO1*, *TOP2A*, *TS*, and *TLE3*). It may be that the inability to maintain integrity at specific DNA loci (i.e., specific genes) leads to metastatic "seeds" that harbor the potential develop metastatic deposits at specific sites. Moreover, it is possible that systemic treatment against metastatic PDAC should target different mechanisms (i.e., DNA repair) than chemotherapy for primary PDAC (i.e., inhibit tumor growth factors) such as the use of PARP inhibitors. Figure 2 demonstrates the frequency of specific molecular alterations that could potentially be used to personalize therapy based on the PDAC disease location.

Gene mutations in *PTEN* are rare and often related to germline mutations whereas molecular variations in expression, such as loss of expression, are more common [20, 21]. Only 1% of all subjects in this study who had *PTEN* molecular alteration found on next-generation sequencing suggesting a possible gene mutation. We do not have data regarding the exact change in sequence but theorize it was likely resulted in a loss of protein expression which is described in the literature in many cancers [22].

Patients with tumors that overexpress *TOPO1* and *TOP2A* may benefit from topoisomerase inhibitors, such as irinotecan, and may, in part, contribute to the generally positive results associated with FOLFIRINOX chemotherapy [23, 24]. Clearly, not all PDAC respond to FOLFIRINOX therapy, and our data suggests that the degree of tumor responsiveness may be related to site of disease for which tumors demonstrate differential molecular alterations. This may fit into the current model of 4 subtypes of PDAC [6] to help understand and develop improved therapies.

While combination chemotherapy, i.e., FOLFIRINOX, has become the standard of care for patients with metastatic PDAC in patients with good performance status, this is a non-personalized approach to systemic chemotherapy. It has been suggested that *ERCC1*, the third most common molecular variant found here, is a predictor of response to cisplatin-based therapy in ovarian malignancies [11, 13, 25]. Our findings suggest that sites of recurrent or metastatic disease are associated with a pattern of molecular alterations that could guide as predictive biomarkers. Oxaliplatin, for instance, is a mainstay of PDAC therapy, as it is included in FOLFIRINOX regimen. Our data support empirical findings that, in large populations overall, patients with metastatic PDAC respond well to FOLFIRINOX-based therapy because many will have liver, lung, and other metastases. There are advantages to utilizing non-personalized multi-agent regimens, such as FOLFIRINOX, as patients are more likely to receive therapy that target at least one or more of their specific molecular alterations. However, the toxicity associated with "non-personalized" combination therapy can be quite severe [26, 27]. A strategy to minimize toxicity upfront [28] would be to dose-reduce the chemotherapy components less likely to be as effective and optimize those components that are expected to yield positive results based on the specific molecular profile of the PDAC tumor. To our knowledge, such a strategy has never been investigated and represents an area of further research.

Our study has a number of limitations. First, this study did not investigate the relationship between survival and site of disease. The relationship between genetic events, molecular alterations, and survival is quite complex in PDAC [9] and reliable survival data was not consistently available for analysis. Our group has investigated genomic instability in other malignancies and we have not identified a consistent relationship suggesting that "more is not worse" with regard to molecular alterations [12]. Furthermore, we cannot state which molecular alterations are passenger and which are driver. A second limitation is that it is possible that tumors labeled as distant pancreatic site are from patients with early stage PDAC (not metastatic or recurrence). This would suggest different tumor biology and a different stage. Due to the de-identified nature of data, we were unable to obtain survival data from the multi-center data set or confirm that all tumor specimens are from recurrent PDAC in the pancreas and not residual primary PDAC. Given that the vast majority of patients will eventually develop metastatic disease, and we are investigating over 2500 subjects, we believe that the particular concern of stage assignment is of limited consequence. A more important limitation is related to the progress of molecular alterations—as PDAC progresses, it is possible that the molecular alterations will increase over time and likely, though not definitively, portend a worse prognosis [29]. Furthermore, we do not have matched data on different sites of disease within the same patient. The lack of treatment and outcome data weakens the assertion that the site of disease recurrence or metastasis is a clinically relevant factor in PDAC.

Conclusion

The site of PDAC metastasis or recurrence is associated with specific patterns of molecular alterations. In particular, aberrant expression (as measured by IHC) or mutation in genes (as determined by next-generation sequencing) associated with DNA maintenance are overrepresented with certain metastatic

PDAC disease sites such as the lung and liver. Given the association between DNA maintenance and chemosensitivity, personalizing therapy for metastatic PDAC based on site of disease and specific molecular alterations is a rational approach that warrants further investigation.

Authors' Contribution Design, patient acquisition, and preliminary discussion: JLD, PVD, MGM, ESG
Writing: MF, MGM, ESG
Critical review and manuscript editing: JLD, PVD, SWB, DS, MGM, ESG
Statistical analysis: LD, JW, ESG
Final approval: all authors

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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