Comprehensive molecular profiling of paired patient samples of primary and metastatic (met) pancreatic ductal adenocarcinoma (PDAC)

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Abstract (#4114)

Background: Pancreatic cancer is the third leading cause of cancer-related death in the U.S. The vast majority of pancreatic cancers are PDAC (90%). Most patients with PDAC die from complications from met disease, thus it is vital to better understand the molecular changes that promote met spread. Prior studies have shown few molecular changes between primary and met PDAC in unpaired analyses, but limited data exist on large-scale comparisons between paired samples from the same patient.

Methods: We analyzed next-generation sequencing (NGS) and immunohistochemistry (IHC) data from 123 patients with multiple PDAC tumors profiled by Caris Life Sciences and compared paired primary and met samples from the same patient. After patients with unrelated second primary cancers were excluded, 113 pairs were used for analysis. McNemar's test was used to compare primary and met tumor pairs.

Results: The most common sites of mets were liver (33%), lung (19%), and peritoneum (18%). The average time between samples was 21.3 months (range 1-92). KRAS status changed in 13.2% of pairs (5.7% gained, 7.5% lost, n = 53, P = 0.710), TP53 status changed in 16.1% (16.1% gained, 0% lost, n = 31, P = 0.025), and SMAD4 status changed in 14.8% (7.4% gained, 7.4% lost, n = 27, P = 1.000). Mets gained expression of TOPO1 (37.5%, n = 80, P = 0.003), TOP2A (42.6%, n = 47, P = 0.003), PTEN (27.3%, n = 66, P = 0.050), and PD-L1 (11.1%, n = 36, P = 0.180). Tumor mutational burden (TMB) increased in mets in 11 of 12 pairs with TMB data (mean 6.67 v. 4.42 mutations per megabase, P = 0.0015 by Wilcoxon signed-rank test).

Conclusion: KRAS mutational status between primary and met PDAC pairs was usually concordant but changed more often than previously reported. TOPO1, TOP2A, and PTEN expression were significantly discordant. The rate of discordance and increase in TMB support profiling of PDAC mets. Continued research into which mutations play key roles in PDAC mets could yield new targets for future therapies.

Introduction

The prevailing thinking about PDAC is that primary and metastatic lesions have similar frequencies of genetic mutations. In addition, metastatic lesions are thought to have the same driver mutations no matter which organ they occupy. The majority of the data in this area are drawn from studies which examine either metastatic lesions alone or in tandem with unpaired analyses of primary tumors. By comparing the molecular profiles of paired patient samples, we hope to further investigate the mutations involved in metastasis of PDAC.

Results

Figure 1. Changes in *KRAS* Status by NGS

Mutational Status	n = 53 (%)
Gained Mutation	3 (6%)
Lost Mutation	4 (7%)
No Change	46 (87%)

Figure 2. Changes in *TP53* Status by NGS

Mutational Status	n = 31 (%)
Gained Mutation	5 (16%)
Lost Mutation	0 (0%)
No Change	26 (84%)

Figure 3. Changes in TOPO1 Status by IHC

Expression Status	n = 80 (%)
Gained Expression	30 (37%)
Lost Expression	11 (14%)
No Change	39 (49%)

Figure 4. Changes in *SMAD4* Status by NGS

Mutational Status	n = 30 (%)
Gained Mutation	3 (10%)
Lost Mutation	1 (3%)
No Change	26 (87%)

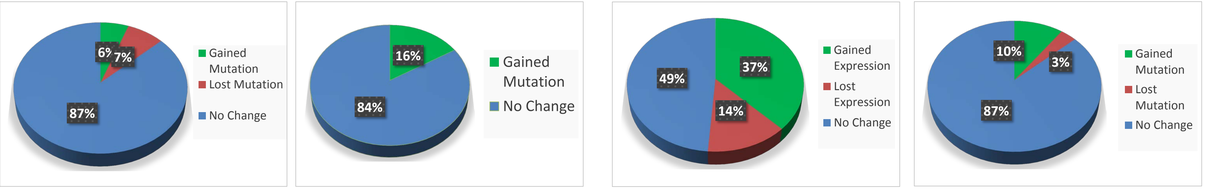

Table 1. Biomarkers tested by IHC, CNV or ISH demonstrating heterogeneity between paired samples

Gene (n, pairs)	Gained	Lost	Unchanged
EGFR (5)	40%	20%	40%
TOP2A (47)	43%	11%	47%
TOPO1 (80)	38%	14%	49%
cMET (16)	38%	13%	50%
TLE3 (17)	29%	18%	53%
PD-1 (11)	9%	36%	55%
PDGFRA (16)	13%	31%	56%
PGP (49)	12%	31%	57%
PTEN (66)	27%	12%	61%
EGFR_ISH (8)	0%	38%	62%
ERCC1 (62)	24%	13%	63%
MGMT (47)	30%	4%	66%
SPARC (57)	7%	23%	70%
TS (88)	16%	14%	70%
MRP1 (24)	17%	13%	71%
RRM1 (80)	14%	4%	82%
cKIT (32)	0%	16%	84%
TUBB3 (43)	11%	3%	86%
PD-L1 (37)	11%	3%	86%
HER2 (69)	9%	4%	87%
ARNT_CNV	0%	7%	93%
MCL1_CNV (14)	0%	7%	93%
MLLT3_CNV (14)	7%	0%	93%
PR (56)	0%	5%	95%
ER (54)	2%	2%	96%

Table 2. Genes tested by NGS demonstrating heterogeneity between paired samples

Gene (n, pairs)	Gained	Lost	Unchanged
PCM1 (10)	10%	20%	70%
USP6 (14)	7%	14%	79%
AFF1 (11)	9%	9%	82%
TP53 (31)	16%	0%	84%
SMAD4 (27)	7%	8%	85%
CEBPA (14)	0%	14%	86%
ERCC5 (14)	0%	14%	86%
MAML2 (14)	0%	14%	86%
PDCD1LG2 (14)	0%	14%	86%
PMS1 (14)	0%	14%	86%
POLE (14)	7%	7%	86%
KRAS (53)	6%	7%	87%
CDKN2A (10)	10%	0%	90%
BCOR (11)	9%	0%	91%
GPR124 (11)	0%	9%	91%
KMT2D (11)	9%	0%	91%
MAP2K4 (11)	0%	9%	91%
BCL11B (12)	0%	8%	92%
BCR (12)	0%	8%	92%
MLLT3 (12)	0%	8%	92%
TERT (12)	0%	8%	92%
ZMYM2 (12)	8%	0%	92%
BARD (13)	8%	0%	92%
BRIP1 (13)	0%	8%	92%
CDC6 (13)	0%	8%	92%
ECT2L (13)	0%	8%	92%
EWSR1 (13)	8%	0%	92%
MN1 (13)	0%	8%	92%
PIK3R1 (13)	8%	0%	92%
TRIP11 (13)	0%	8%	92%
TRRAP (13)	8%	0%	92%
ABL2 (14)	7%	0%	93%
AFF3 (14)	0%	7%	93%
ALK (14)	7%	0%	93%
AMER1 (14)	7%	0%	93%
ASXL1 (14)	0%	7%	93%
CACNA1D (14)	0%	7%	93%
CCND3 (14)	0%	7%	93%
DDIT3 (14)	0%	7%	93%
DDX5 (14)	0%	7%	93%
ELK4 (14)	7%	0%	93%
EP300 (14)	0%	7%	93%
ETV5 (14)	7%	0%	93%
HOXD11 (14)	7%	0%	93%
IRS2 (14)	7%	0%	93%
KEAP1 (14)	7%	0%	93%
LRP1B (14)	7%	0%	93%
NCL1 (14)	7%	0%	93%
NUP214 (14)	0%	7%	93%
PBRM1 (14)	0%	7%	93%
PDGFRB (14)	7%	0%	93%
RNF43 (14)	7%	0%	93%
TNFAIP3 (14)	7%	0%	93%
TSC1 (14)	7%	0%	93%
RB1 (29)	7%	0%	93%
BRCA2 (19)	0%	5%	95%
PTEN (27)	0%	4%	96%
STK11 (28)	4%	0%	96%
APC (30)	3%	0%	97%
ATM (30)	0%	3%	97%
NOTCH1 (30)	3%	0%	97%
FLT3 (31)	3%	0%	97%
JAK2 (31)	3%	0%	97%
CSF1R (32)	3%	0%	97%
GNAS (32)	3%	0%	97%

Table 3. Changes in TMB in mutations per megabase (mt/Mb)

Pair	1st (per Mb)	2nd (per Mb)	Net % Change	Time Between Profiles(mos)
1	4	6	150%	14
2	2	6	300%	24
3	4	6	150%	14
4	4	6	150%	13
5	4	6	150%	13
6	4	6	150%	3
7	4	4	100%	9
8	2	4	200%	4
9	6	12	200%	5
10	4	5	125%	3
11	4	6	150%	46
12	9	8	89%	16

Highlighted Examples of Matched Pairs

	Biomarker	16-Dec	17-May	Status
Next Generation Sequencing	Tumor Mutation Burden	6 mt/Mb	12 mt/Mb	doubled mutation burden
	Microsatellite Instability	Stable	Stable	no change
	KRAS	wildtype	W36R, T133R	gained mutation
	KRAS	wildtype	G12D	gained mutation
	ARID1	wildtype	L311F	gained mutation
	BARD1	wildtype	D458H	gained mutation
	BUB1B	E409D	E409D	no change
	ELK4	wildtype	L353F	gained mutation
	ERCC4	P379S	P379S	no change
	FANCA	P201S	P201S	no change
	FANCD2	Q26H	Q26H	no change
	IL2B	T97M	T97M	no change
	KMT2D	wildtype	N3375S	gained mutation
	LRP1B	wildtype	D3600G, C3601S	gained mutation
	MRE1	R560Q	R560Q	no change
	MLLT3	S167dup	wildtype	lost mutation
	NUP214	I765V	wildtype	lost mutation
	PALB2	L1143H	L1143H	no change
	PIGM1G	E754K	E754K	no change
	SMAD4	wildtype	S417X	gained mutation
	TET1	I1778M	I1778M	no change
	TP53	wildtype	H179P	gained mutation
	TRAF7	V156I	V156I	no change
	ZNF217	E914_P915delinsDS	E914_P915delinsDS	no change
Copy Number	APC	2.50	0.50	lost gene dosage
	IGF1R	0.90	3.50	gained gene dosage
	NTRK3	0.72	4.12	gained gene dosage
Immunohistochemistry	ERCC1	0+ 100%	2+ 20%	gained expression
	RRM1	0+ 100%	1+ 20%	gained expression
	TOPO1	0+ 5%	2+ 30%	gained expression
	TS	0+ 100%	1+ 15%	gained expression
	TUBB3	2+ 20%	1+ 5%	lost expression
	PDL1 (SP142)	0+ 100%	0+ 100%	no change
	MLH1	1+ 50%	1+ 50%	no change
	MSH2	1+ 50%	1+ 50%	no change
	MSH6	1+ 50%	1+ 40%	no change
	PMS2	1+ 50%	1+ 50%	no change

Table 4. 58 year-old female, lifelong non-smoker with pancreatic adenocarcinoma. Profiling results are shown from the initial biopsy of head of pancreas which occurred in Dec. 2016 and biopsy of metastatic disease in para-aortic lymph nodes, 5 months later in May 2017.

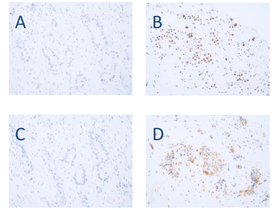

Figure 5. IHC staining of biomarkers that change expression between primary and metastatic disease (A: TOPO1 primary, B: TOPO1 met, C: PD-L1 sp142 primary, D: PD-L1 sp142 met)

Conclusions

- KRAS mutational status between primary and met PDAC pairs changed more often than previously reported
- TOPO1, TOP2A, and PTEN expression between primary and met PDAC pairs were significantly discordant
- There was a significant increase in TMB in PDAC mets
- The discordance in mutational status and TMB between primary and met PDAC pairs supports the molecular profiling of PDAC mets
- Further research into which mutations play key roles in the metastasis of PDAC could yield new targets for future therapies

References

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