Molecular characterization of appendiceal cancer and comparison with right-sided (R-CRC) and left-sided colorectal cancer (L-CRC)

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Abstract ID: 3611

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Abstract

Background: The natural history and prognosis of appendiceal adenocarcinomas (AA) differ from that of adenocarcinomas arising in other large bowel sites. Compared to colorectal cancer (CRC), AA has more peritoneal dissemination and worse outcome. Only a few reports exist on molecular differences between AA and CRC.

Methods: A total of 183 samples from AA (46 adenocarcinoma, NOS (NOS), 66 pseudomyxoma peritonei (PMP), 44 mucinous (MU), 27 signet ring (SR) and), 994 samples from right-sided CRC (R-CRC), and 1080 from left-sided CRC (L-CRC) were tested with Next-Generation Sequencing (NGS) on a 592-gene panel and immunohistochemistry (IHC). Microsatellite instability (MSI) and tumor mutational burden (TMB) were tested by NGS, and PD-L1 (SP142) by IHC. Statistical comparisons of AA and R-and L-CRC were done by Fisher's exact test.

Results: High mutation rates in AA were seen in *KRAS* (55%), *TP53* (40%), *GNAS* (31%), *SMAD4* (16%), *APC* (10%), *ARID1A* (8%), *RNF43* (7%), *PIK3CA* (6%) and *BRAF* (5%). MSI-high was seen in 2.2%, TML-high (>= 17mut/MB) in 2.2% and PD-L1 expression in 2.8%. When compared to both R- and L-CRC, AA showed significantly higher mutation rates of *GNAS, SMAD4* and lower *TP53*, *APC*, *PIK3CA*, *FBXW7*, *NRAS*, and *AMER1* ($p < 0.05$). Alterations associated with immune checkpoint inhibitor responses (MSI-high, TML-high, PD-L1) showed similar frequency in AA compared to L-CRC, but not R-CRC. Histopathological subtypes of AA showed different molecular patterns: PMP carried the highest *KRAS* (74%), *GNAS* (63%) and no *BRAF* mutations. MU was characterized by *GNAS* mutation rate of 25%. SR showed the lowest *KRAS* (15%), *PIK3CA* (0%), and *APC* (0%) mutation rates. PMP had no MSI-high or TML-high cases.

Conclusions: Molecular characterization of AA revealed different characteristics compared with CRC; similarities were observed between AA and L-CRC despite anatomical distance, and molecular heterogeneity among histological subtypes were seen. These molecular differences may be critical to develop new treatments for appendiceal adenocarcinoma.

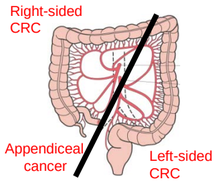
Background

・The natural history and prognosis of appendiceal adenocarcinomas (AA) differ from that of adenocarcinomas arising in other large bowel sites. [1]

・Compared to colorectal cancer (CRC), AA has a clinical feature which has more peritoneal dissemination and poorer prognosis.

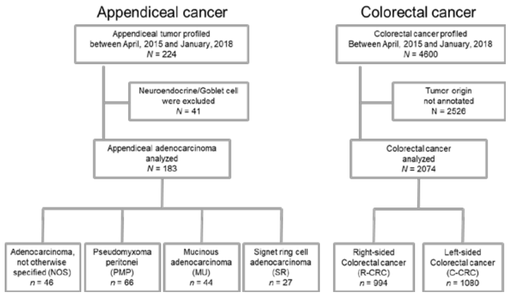
・In AA, the prognosis varies according to histologic type and tumor stage.[2] but the association between molecular change and prognosis is not well known.[3]

・In CRC, genetic or epigenetic expression profiling has suggested phenotypic clustering with different outcomes.



・We thus examine the genomic profiling of AA and comparison with right-sided (R-CRC) and left-sided CRC (L-CRC), using integrated data within a total of 183 samples from AA, 994 from R-CRC, and 1,080 from L-CRC.

Study design



Cases submitted to a commercial CLIA-certified laboratory (Caris Life Sciences) from April of 2015 through January of 2018 were retrospectively analyzed.
Samples were profiled by next-generation sequencing (NGS) and immunohistochemistry (IHC) using Caris Molecular Intelligence.

Method

Next-Generation Sequencing (NGS)
NGS was performed: a custom-designed SureSelect XT assay was used to enrich 592 whole-gene targets (Agilent Technologies, Santa Clara, CA).

Microsatellite instability (MSI)
MSI was examined using over 7,000 target microsatellite loci and compared to the reference genome hg19 from the University of California, Santa Cruz (UCSC) Genome Browser database. The number of microsatellite loci that were altered by somatic insertion or deletion was counted for each sample. Only insertions or deletions that increased or decreased the number of repeats were considered. Genomic variants in the microsatellite loci were detected using the same depth and frequency criteria as used for mutation detection.

Tumor mutation burden (TMB)
TMB was measured by counting all non-synonymous missense mutations found per tumor that had not been previously described as germline alterations (592 genes and 1.4 megabases [MB] sequenced per tumor). The threshold to define TMB-high was greater than or equal to 17 mutations/MB.

Immunohistochemical (IHC) Analysis
IHC staining was scored for intensity (0 = no staining; 1+ = weak staining; 2+ = moderate staining; 3+ = strong staining) and staining percentage (0-100%). Results were categorized as positive or negative based on published clinical literature.

Statistical analysis
All statistical analyses were performed with SPSS v23 (IBM SPSS Statistics, Cary, USA), and all tests were two sided at a significant level of 0.05. The comparisons of molecular profiles between groups were analyzed using Fisher's exact test. Cases with missing information in any of the categorical data were not included in the analysis.

Results

Table 1 Patients characteristics

Samples	AA and CRC					AA				
	AA N = 183	R-CRC N = 994	L-CRC N = 1080	*P* value AA vs. R-CRC	*P* value AA vs. L-CRC	NOS n = 46	PMP n = 66	MU n = 44	SR n = 27	*P* value
Age (median, range)	56(22-83)	63(16-95)	58(18-91)	<0.0001	0.1201	56.5(22-83)	56(30-83)	61(25-82)	54(32-76)	0.7825
Sex (male/female)	77/106	497/497	554/526	0.0535	0.0208	18/28	29/37	20/24	10/17	0.8618
Location (primary/metastatic/unknown)	50/133/0	744/332/4	735/255/4	<0.0001	<0.0001	15/31/0	15/51/0	11/33/0	9/18/0	0.5808

AA, appendiceal adenocarcinoma; L-CRC, left-sided colorectal cancer; MU, mucinous; NOS. adenocarcinoma, not otherwise specified; PMP, pseudomyxoma peritonei; R-CRC, riight-sided; SR, signet ring.

Table 2 Frequency of gene mutations by NGS.

Gene	AA N = 183	R-CRC N = 994	L-CRC N = 1080	*P* value	*P* value
	%	%	%	AA vs. R-CRC	AA vs. L-CRC
GNAS	31	2*	1*	<0.01	<0.01
SMAD4	16	11*	10*	0.04	0.03
TP53	40	66*	75*	<0.01	<0.01
APC	10	70*	83*	<0.01	<0.01
PIK3CA	6	22*	17*	<0.01	<0.01
FBXW7	3	11*	9*	<0.01	0.01
NRAS	1	3*	5*	0.04	<0.01
AMER1	0	9*	2*	<0.01	0.02
ARID1A	8	26*	19	<0.01	
BRAF	5	17*	5	<0.01	
ATM	2	7*	5	0.01	
KMT2D	2	7*	2	<0.01	
PTEN	1	8*	3	<0.01	
MSH6	1	5*	2	0.02	
HNF1A	1	5*	1	0.02	
PTCH1	1	5*	1	<0.01	
CTNNB1	0	4*	1	<0.01	
KRAS	55	56	43*		<0.01
RNF43	7	11	2*		<0.01

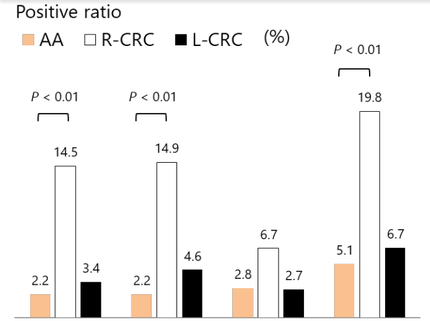
Blanks are p > 0.05 * *P* < 0.05 compared to AA

Table 3 Frequency of gene mutations by NGS in appendiceal adenocarcinoma.

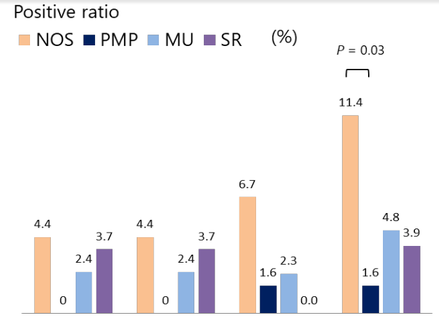
Gene	NOS n = 46	PMP n = 66	MU n = 44	SR n = 27	*P* value	*P* value	*P* value
	%	%	%	%	NOS vs. PMP	NOS vs. MU	NOS vs. SR
KRAS	44	74*	64	15*	<0.01		0.02
GNAS	7	63*	25*	4	<0.01	0.02	
TP53	51	23*	57	33	<0.01		
SMAD4	15	15	20	11			
RNF43	9	6	7	4			
APC	22	2*	16	0*	<0.01		0.01
PIK3CA	15	2*	7	0*	<0.01		0.04
BRAF	7	0	9	7			
ARID1A	11	0	15	11			

* *P* < 0.05 compared to NOS

Figure 1 Immune profile of AA and comparison with R-CRC and L-CRC



MSI	n	AA	n	R-CRC	n	L-CRC
MSI	181	2.2%	970	14.5%	1055	3.4%
TML	179	2.2%	961	14.9%	1049	4.6%
PD-L1	178	2.8%	946	6.7%	1033	2.7%
Any one	175	5.1%	916	19.8%	1003	6.7%



MSI	n	NOS	n	PMP	n	MU	n	SR
MSI	45	4.4%	66	0%	42	2.4%	27	3.7%
TML	45	4.4%	65	0%	42	2.4%	27	3.7%
PD-L1	45	6.7%	63	1.6%	44	2.3%	26	0.0%
Any one	44	11.4%	63	1.6%	42	4.8%	26	3.9%

Study Highlights

- ✓ High mutation rates in AA were seen in *KRAS* (55%), *TP53* (40%), *GNAS* (31%), *SMAD4* (16%), *APC* (10%), *ARID1A* (8%), *RNF43* (7%), *PIK3CA* (6%) and *BRAF* (5%).
- ✓ Immune profile of AA is similar to L-CRC but not R-CRC.
- ✓ In AA, PMP exhibited higher mutation rate in *KRAS* (74%) and *GNAS* (63%), and lower mutation rate in *TP53* (23%), than other histopathological types of appendiceal adenocarcinoma, and no *BRAF* mutations.
- ✓ PMP had no MSI-high or TML-high cases

Conclusion

Molecular characterization of appendiceal cancer revealed different profiles from colorectal cancer. GNAS might be a clinical target for appendiceal cancer, especially for pseudomyxoma peritonei.

References

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