

The Mutational Landscape of Upper Gastrointestinal Adenocarcinomas- A Study of Similarities and Differences

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Abstract

Purpose-

The gastrointestinal tract is home to a wide variety of neoplasms. Gastrointestinal adenocarcinomas display distinct prognostic patterns. With the advent of next generation sequencing, attempts are being made to delineate distinct molecular characteristics of these adenocarcinomas from adjoining anatomical sites.

Methods-

Thirty-seven cases of upper gastrointestinal adenocarcinomas including those of the esophagus, gastroesophageal junction, stomach, small intestine and gallbladder were retrieved. Next generation sequencing data consisting of base substitutions, copy number variations, indels and rearrangements, in 324 genes, were analyzed for recurrent genetic abnormalities. Statistical analysis was performed using IBM SPSS25 and SAS software.

Results-

Genetic alterations were found in 181 genes. APC mutations were found in 50% of the esophageal adenocarcinomas, 5% of the gastric adenocarcinomas and 33.3% of the small intestinal adenocarcinomas ($p=0.04$). PIK3 gene family mutations were found in 10% of the gastric adenocarcinomas, 66% of the gall bladder adenocarcinomas and 66% of the small intestinal adenocarcinomas ($p=0.002$). The mutations were found exclusively in the PIK3 class 1 family.

Conclusion-

In this study, APC gene mutations were found to be more frequent in esophageal and small intestinal adenocarcinomas than previously reported. PIK3 class 1 gene family mutations were found to be more frequent in gallbladder and small intestinal adenocarcinomas.

Introduction

The gastrointestinal tract is home to a wide variety of vastly different, diagnostically challenging neoplasms.¹ When taken as a whole, adenocarcinomas of the gastrointestinal tract are one of the most common malignancies in the world.² Though they share morphological similarities, these gastrointestinal adenocarcinomas display distinct clinical characteristics in terms of different risk factors and discrete prognostic patterns.³⁻⁵

Owing to their morphological heterogeneity, the adenocarcinomas of the gastrointestinal tract are difficult to profile based on location.^{5,6} Over the past two decades, immunohistochemistry has evolved and has been routinely incorporated into the workup and assessment of adenocarcinomas of the gastrointestinal

tract.⁵ Attempts have been made to utilize immunohistochemical profiles for determining various tumor characteristics including the origin of a malignancy in the setting of widely metastatic disease.^{5,6}

Multiple studies such as those summarized in a review article by Wong et al emphasize the extremely high specificity of the CK7-/CK20 + immunoprofile for colorectal cancers that effectively separates them from other gastrointestinal adenocarcinomas.⁷ However, the upper gastrointestinal adenocarcinomas including esophageal, gastroesophageal, gastric, small intestinal and gallbladder adenocarcinomas predominantly express CK7 and not CK20.⁷ Other commonly used markers such as CDX2, Villin, CK17 and MUCs exhibit heterogenous staining patterns and do not aid in differentiating between these neoplasms.^{5,8-10}

With the advent of molecular techniques and next generation sequencing, attempts are being made to delineate distinct molecular characteristics of these morphologically related adenocarcinomas from adjoining anatomical sites.^{4,11-14} Despite these efforts the molecular biology of upper gastrointestinal adenocarcinoma, still requires further elucidation. Identifying and understanding the distinct molecular pathways holds the key to finding novel diagnostic and therapeutic targets for these tumors which usually present at advanced stages which portends a poor prognosis.

Materials And Methods

This study was reviewed and approved by the Institutional Review Board and Ethics Committee of Mount Sinai Medical Center.

The aim of the present study was to investigate the clinical role and significance of a panel of genetic alterations found in adenocarcinoma cases retrieved from our pathology records.

Selection of tumors for tissue profiling

Only adenocarcinomas in which the primary site of the tumor could be established by clinical and radiological findings, were included in this study. Cases with history of another malignancy or concomitant second primary were excluded from the study.

Genetic testing and data analysis

Formalin fixed paraffin embedded tissue blocks of the tumor were selected and sent for next generation sequencing which was performed at an outside institution. The data collected from these sequencing studies included the total number and type of genetic alterations including base substitutions, copy number variations, indels and rearrangements. The obtained sequencing data was analyzed for recurrent genetic abnormalities.

After the statistically significant genetic abnormalities were detected for the adenocarcinoma cases, the data was stratified by primary sites of tumor location mentioned above. In the stratified data, each

primary site of tumor location (each category) showed the presence or absence of pathological diagnosis for that specific site of tumor location. This was necessary to make binary logistic regression models for each primary site of tumor location.

Five binary logistic regression models were done for the five primary sites of tumor locations included in the study. This was done in order to screen for a possible association between the significant genetic abnormalities (PIK3 and APC) and the specific primary sites of tumor locations. The logistic regression models used the presence or absence of statistically significant genetic alterations as the covariate and presence or absence of pathological diagnosis (in the different primary sites of tumor locations) as the dependent variable.

Statistical analysis.

Statistical analyses were performed using IBM SPSS25 and SAS software. The study included only non-parametric data which was compared using Fisher Exact and chi square test for statistical significance. A *p*-value less than 0.05 was considered statistically significant.

Results

Thirty-seven cases of adenocarcinoma were included in this study. Of these, six were of esophageal origin, two of gastroesophageal junction origin, twenty of gastric origin, six of small intestinal origin and three were primaries from the gallbladder. The study sample consisted of 11 female patients between the ages of 40 to 87 years old and 26 male patients between the ages of 34 to 96 years old. The mean (66.2) and SD (14.6) of the age distribution lead to the normal range 37–95.4 years.

There was no significant difference between the age or sex distribution among the various groups (Table 1. Characteristics of study cases). Genetic alterations including base substitutions, insertions, deletions, copy number alterations and rearrangements were found in a total of 181 genes in the 37 cases studied (Table 2. List of genes found to be altered in all the cases of adenocarcinoma included in the study).

Table 1
 Characteristics of study cases.

Characteristic	Esophagus	GE junction	Stomach	Gallbladder	Small Intestine
Age (Mean)	72.3	61	66.7	64.6	61.3
Gender (M:F)	6:0	2:0	14:6	1:2	3:3
APC mutation	3	0	1	0	2
TP53 mutation	3	1	9	2	3
PIK3mutation	0	0	2	2	4
Sample size	6	2	20	3	6
*GE junction = Gastroesophageal junction					

Table 2

List of genes found to be altered in all the cases of adenocarcinoma included in the study.

ABL1	CDK1	FAT1	IDH2	MSH3	PPP2R1A	TNFAIP3
ABL2	CDK2	FBXW7	INHBA	MSH6	PRD1	TOP2A1
AKT2	CDK8	FGF3	INPP46	MTOR	PRDC	TP53
APC	CDKN2A	FGF4	IRF4	MYCL1	PREX2	TSC1
AR	CDKN2B	FGF10	IRS2	MYS3	PRKAR1A	TSC2
ARAF	CDX1	FGF19	JAK1	NF1	PRKC1	U2AF1
ARID1A	CDX2	FGFR1	JAK2	NKX2	PRKCDC	VEGFA
ARID1B	CEBPA	FGFR2	JAK3	NITCH1	PTCH1	VHL
ARID2	CHD1	FGFR3	KDM5A	NOTCH2	PTEN	WISP3
ASXL1	CHD2	FGFR4	KDR	NOTCH3	PTPN11	WT1
ATM	CHD4	FLT1	KEAP1	NRAS	QK1	XPO1
ATR	CHEK2	FLT3	KIT	NTRK3	RANBP2	ZBTB
ATRX	CKIT	FLT4	KRAS	NTSC2	RICTOR	ZNF217
AXIN1	CREBBP	FOXP1	L194R	PANCA	RMB10	ZNF703
BAP1	CSF1R	FRS2	LRP1B	PARK2	RNF43	TNFAIP3
BCOR	CTCF	GATA3	MAG12	PAX5	ROS1	TOP2A1
BCORL1	CTNNB1	GATA4	MAPK3KI	PBRM1	SDHD	TP53
BLM	CYLD	GATA6	MDM2	PDGFRA	SF3B1	TSC1
BRAF	DDR2	GF2	MDM4	PDGFRB	SMAD2	TSC2
BRCA2	EGFR	GLI1	MED12	PIK3CA	SMAD3	
BRD4	EPHA3	GNAS	MEF2B	PIK3CB	SMAD4	
BTK	ERBB2	GPR124	MLH1	PIK3CG	SMARCA4	
CARD2	ERBB3	GRM3	MLL1	PIK3R1	SNCAIP	
CARD11	ESR1	HNF1A	MLL2	PIK3R2	SOX9	
CBL	EWSR1	HSD3B1	MLL3	PLCG2	SPEN	
CCNE1	FANCC	HSP90AA1	MPL	POLD1	SPTA1	
CD70	FANCD2	IDH1	MSH2	POLE	SRC	

APC mutations were found in 15.7% of the total adenocarcinoma cases included in the study. APC mutations were found 50% of the esophageal adenocarcinomas, 5% of the gastric adenocarcinomas and 33.3% of the small intestinal adenocarcinomas. None of the gastroesophageal junction adenocarcinomas or gallbladder adenocarcinomas had APC mutations. This difference in the frequency of APC mutations in adenocarcinomas was significant with a p-value of 0.04. The mutations were not limited to any particular site of the APC gene.

Of the 37 adenocarcinomas, 48.6% had TP53 mutations. TP53 mutations were found in 66.7% adenocarcinomas of gallbladder, 50% adenocarcinomas of esophagus and gastroesophageal junction, 40% gastric adenocarcinomas and 33.3% small intestinal adenocarcinomas. There was no significant difference in the TP53 mutational frequency between the different locations. Only two of the tumors (one gastric and one small intestinal) had concurrent APC and TP53 mutations. The remainder of the TP53 mutant adenocarcinomas were APC wild type. (p = 0.2)

PIK3 gene family mutations were found in 22.2% of the total adenocarcinoma cases included in the study. PIK3 gene family mutations were found in 10% of the gastric adenocarcinoma, 66% of the gallbladder adenocarcinomas and 66% of the small intestinal adenocarcinomas. This difference in the frequency of PIK3 mutations in adenocarcinomas was significant with a p-value of 0.002. The mutations were found exclusively in PIK3 class 1 family in all cases; PIK3CA was mutated in two small intestinal and one gastric adenocarcinoma, PIK3CB was mutated in one small intestinal and one gastric adenocarcinoma, PIK3CG was mutated in one small intestinal adenocarcinoma and PIK3R1/R2 in the two gallbladder adenocarcinomas. Of the 8 PIK3 mutant tumors in this study, only two (both small intestinal) adenocarcinomas had concomitant APC mutations. The remainder PIK3 mutant tumors were APC wild type.

The logistic regression model for the small intestine primary tumor location was the only model that satisfied the convergence criterium and that was statistically significant with a concordance-statistic value of 0.76 (Table 3. Association of predicted probabilities and observed responses for the small intestine site of tumor location model). The Wald Chi-Square test, the Likelihood Ratio Chi-Square test and the Score Chi-Square Test were all statistically significant with p-values of 0.03, 0.02 and 0.011 respectively (Table 4. Testing global null hypothesis for association between APC and PIK3 genetic abnormalities and small intestine site of tumor location). The model also shows the analysis of maximum likelihood estimates which was significant for the PIK3 gene family mutation with a p-value of 0.01 but not for the APC mutation (Table 5. Analysis of maximum likelihood estimates for the small intestine site of tumor location model). Also, statistically significant values were found for the PIK3 mutation in the 95% Wald confidence interval (CI): 1.63–96.2, but not for the APC mutation (Table 6. Odds ratio estimates for the small intestine site of tumor location model).

Table 3
Association of predicted probabilities and observed responses for the small intestine site of tumor location model.

Percent Concordant	62.2	Somers' D	0.533
Percent Discordant	8.9	Gamma	0.750
Percent tied	28.9	Tau-a	0.152
Pairs	180	c	0.767

Table 4
Testing global null hypothesis for association between APC and PIK3 genetic abnormalities and small intestine site of tumor location (Beta = 0).

Test	Chi-Square	DF	Pr > ChiSq
Likelihood Ratio	7.7306	2	0.0210
Score	8.9739	2	0.0113
Wald	6.4754	2	0.0393

Table 5
Analysis of maximum likelihood estimates for the small intestine site of tumor location model.

Parameter	DF	Estimate	Standard error	Wald Chi-Square	Pr > ChiSq
Intercept	1	-2.7906	0.8140	11.7535	0.0006
APC	1	1.0792	1.1944	0.8164	0.3662
PIK3	1	2.5304	1.0389	5.9329	0.0149

Table 6
Odds ratio estimates for the small intestine site of tumor location model.

Effect	Point estimate	95% Wald Confidence limits
APC	2.942	0.283 30.576
PIK3	12.559	1.639 96.209

Discussion

The gastrointestinal tract includes the luminal organs extending from the esophagus to the rectum in addition to the pancreas and the gall bladder.¹⁵ In addition to the sizeable cellular mass, the epithelium has a rapid turnover, laying the foundation for gastrointestinal cancers that are among the most frequent malignancies resulting in mortality.¹⁶ These tumors usually present late with metastatic disease. Though

morphologically similar, adenocarcinomas of different sites in the gastrointestinal tract are clinically distinct with unique risk factors and dissimilar prognostic behavior.^{3–5}

The determination of the primary site of these adenocarcinomas within the luminal GI tract in the setting of widely metastatic disease has remained challenging despite the advances in immunohistochemistry.^{5,6} The CK7-/CK20 + immune-profile has good specificity for differentiating colorectal adenocarcinoma from other gastrointestinal adenocarcinomas.^{7,17–18} Similarly, pancreatic ductal adenocarcinomas display a CK7+/CK20 + immune-profile along with substantially higher CK17 expression.^{19–20}

But when it comes to the upper gastrointestinal adenocarcinomas including those of the esophagus, gastroesophageal junction, stomach, small intestine and gallbladder, immunohistochemical profiles are indistinct and overlapping. CK7 is usually positive in most of them while CK 20 is usually negative.⁷ Other markers such as CDX2, Villin, CK17 and MUCs do not display specific staining patterns and aren't helpful in differentiating these adenocarcinomas when used individually or as a panel.^{5,8–10,21}

The emergence of high throughput technologies such as next generation sequencing technology has heralded the beginning of the genomic era. Novel genomic and epigenomic biomarkers and signatures are being discovered and developed for early detection and prognosis of gastrointestinal cancers.²² In addition, scientists are endeavoring to understand how different molecular events lead to varying biologic properties and clinical features of these cancers based on different cells and tissues of origin.²³

Morphologically similar tumors with similar immunohistochemical profiles arising in different organs may be from identical preceding events driving the tumorigenesis. However, even morphologically identical tumors arising in different organs differ substantially not only in terms of the oncogenic threats and environmental risk factors but also in cellular dynamics and tumorigenic potential. These inter-tumor dissimilarities, when identified, can not only improve diagnostic accuracy but also identify therapeutic targets and further patient welfare using precision medicine.²³

A comprehensive comparative analysis of genetic alterations identified by high throughput sequencing can potentially uncover tissue specific determinants in different gastrointestinal adenocarcinomas which can translate to differences in prognosis and may help direct therapeutic decisions. Presence or absence of certain gene mutations and/or varying mutational frequency result in tissue specific mutational signatures.²³

Beta-catenin, a protein coded by the CTNNB1 gene, is integral in intercellular adhesion and signal transduction and its degradation is regulated largely by adenomatous polyposis coli (APC) gene. Mutation in either of these can cause aberrant accumulation of beta catenin leading to increased transcription of downstream target proteins of the wingless integration site family member (WNT) signaling pathway such as MYC and CCND1.²⁴ The dysregulation of the APC/beta-catenin and WNT signaling pathway is an integral mechanism of tumorigenesis in several cancers, most prominently in

colorectal carcinomas.²⁵⁻²⁷ Choi et al reported a very low frequency of APC/beta catenin mutations in their study analyzing esophageal and esophagogastric junction adenocarcinomas based on partial screening mutational analyses.²⁷

In our study, APC gene mutations were found to be most frequent in esophageal adenocarcinomas followed by small intestinal adenocarcinomas and infrequent in gastric adenocarcinomas. Choi et al reported similar findings in esophageal adenocarcinomas in their study in 97 tumors.²⁷ Salem et al reported similarly low frequency of APC mutations in gastric adenocarcinomas but found significantly lower frequency of APC mutations in esophageal adenocarcinomas.¹⁴ The reported frequency of APC mutations in gastric adenocarcinomas varies widely. Fang et al reported an APC mutation frequency of 25% in gastric adenocarcinomas.²⁸ Rokutan et al in their study of 43 gastric intramucosal adenocarcinomas found a higher frequency of APC mutations than in our study.²⁹ However, they reported that the APC and TP53 mutations were mutually exclusive. This is reflected in our study where 88.9% of the TP53 mutated adenocarcinomas were APC wild type though this finding did not reach statistical significance. This might be attributed to the low number of study subjects and might reach statistical significance if a higher number of cases were evaluated supporting the existence of TP53/APC molecular subsets. Similar to gastric adenocarcinomas, wide variations have been reported in APC gene mutations in small intestinal adenocarcinomas. Schrock et al found the APC mutational frequency to be around 26% in 317 small intestinal adenocarcinomas studied.³⁰ Similar findings were reported by Hanninen et al.³¹ However, in a recent study, Ota et al reported a much higher frequency of APC mutations in small intestinal adenocarcinomas.³² Our findings are comparable to the APC mutational frequency reported by Ota et al. The difference in APC mutational frequency among different studies may be ascribed to locational differences. The reported incidence of APC mutation in duodenal adenocarcinomas is much lower as compared to other small intestinal locations.³² The majority of adenocarcinomas in the study by Schrock et al were duodenal.³⁰ However, this cannot be ascertained as the small intestinal adenocarcinomas were not further stratified based on location in our study due to the small number of small intestinal adenocarcinomas included. None of the gastroesophageal junction adenocarcinomas or gallbladder adenocarcinomas included in this study had any mutations in the APC gene and this low incidence is similar to those reported previously.^{27,33}

PIK3 gene mutations were found to be relatively more frequent in small intestinal and gall bladder adenocarcinomas as compared to esophageal and gastric adenocarcinomas. All the PIK3 gene family mutations were limited to class 1 PIK3 genes with the majority localizing to PIK3CA as reported in previous studies.³⁴⁻³⁸ PIK3CA mutations are reported to occur in 8-10% cancers.³⁹ Disturbances in the PIK3 signaling pathway and its regulation are known to underlie numerous human diseases. Activating mutations in the genes encoding the catalytic subunits of class IA PIK3 have been reported in several cancer types.⁴⁰ The frequency of PIK3 mutations in esophageal and gastric adenocarcinomas has been reported to be low in multiple studies as reflected in our study.^{14,34-35}

The previously reported frequency of PIK3 gene family mutations in gallbladder adenocarcinoma is much lower than found in our study.^{36,37} Though, this could represent a sampling bias due to the small number of gallbladder adenocarcinoma cases included in this study, a more plausible explanation for the higher than reported frequency of PIK3 gene family mutations is the inclusion of mutations in all the class 1 genes. The mutations in the PIK3 gene family in gallbladder adenocarcinomas were limited to the class 1 regulatory subunit 1 and 2 genes. All class IA catalytic subunits interact and are controlled by regulatory subunits, and mutations/deletions in these regulatory subunits have been identified in multiple cancers.⁴⁰ Though the role of PIK3R1 and PIK3R2 mutations in gallbladder adenocarcinoma has so far not been described, they are known oncogenic drivers in endometrial adenocarcinoma where gain of function mutations in PIK3R2 results in oncogenesis via PTEN stabilization.^{41,42}

Again, like for gallbladder adenocarcinoma, the frequency of PIK3 family mutations in small intestinal adenocarcinoma was found to be much higher in our study when compared to previously published data.^{30-31, 38} The majority of these mutations were in PIK3CA as previously reported however mutations were also found in PIK3CG and PIK3CB genes accounting for the higher than reported incidence. Though the incidence of PIK3CA mutation in intestinal adenocarcinomas is reportedly low, the PI3K/AKT pathway is the most mutated pathway, where at least one gene was mutated in the majority of small intestinal adenocarcinomas.³¹ Hare et al in their study showed that the most common PIK3CA mutation (Pik3caH1047R seen in colorectal carcinomas), when expressed at physiological levels, is insufficient to initiate intestinal tumorigenesis. However, when acting in tandem with APC loss, it promotes the development of invasive adenocarcinomas in the small intestine.⁴³ This tandem effect is seen on the logistic regression model in small intestinal adenocarcinomas in our study, though the statistical significance is limited by the small study set.

In addition to the small study population that remains a limitation of this study, the association of different mutations with the histo-morphological types of adenocarcinomas was not assessed. Also, the association between the various mutations and presence or absence of precursor lesions was not assessed.

Conclusions

In this study on luminal upper gastrointestinal adenocarcinomas, APC gene mutations were found to be more frequent in esophageal and small intestinal adenocarcinomas than previously reported. PIK3 class 1 gene family mutations, when taken as a group, were found to be more frequent in gallbladder and small intestinal adenocarcinomas. Though a majority of the cases had mutations in PIK3CA, mutations in other genes of the PIK3 class 1 family, namely PIK3CB, PIK3CG, PIK3R1 and PIK3R2 were also identified in a subset of cases.

Declarations

1. **Funding-** Nil.

2. **Conflicts of interest/ competing interest-** The authors declare they have no conflicts of interest.
3. **Ethics approval-** This study was approved by the IRB at MSMC, FL (FWA00000176).
4. **Consent to participate-** Not applicable (retrospective data analysis)
5. **Consent to publish-** Not applicable (retrospective data analysis)
6. **Availability of data and material-** Not applicable.
7. **Code availability-** Not applicable.
8. **Author contributions-** All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Kritika Krishnamurthy and Sophia Navajas Urioste. The first draft of the manuscript was written by Kritika Krishnamurthy and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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