

Table 1: Pathology of Duodenal Specimens from FAP Cases and FAP Controls

FAP Cases				FAP Controls		
Cancer Patient (CP#)	Cancer Location	Adenoma Histology + Degree of Dysplasia	Most Recent Spigelman Stage	Non-Cancer Patient (NCP#)	Adenoma Histology + Degree of Dysplasia	Spigelman Stage
CP1	Duodenum	TA + LGD	III	NCP1	TA + LGD	II
CP2	Ampullary	TA + LGD	III	NCP2	TVA + LGD	III
CP3	Ampullary	TA + LGD	0 ^a	NCP3	TA + LGD	II
CP4	Duodenum	VA + LGD	III	NCP4	TVA + LGD	III
CP5	Ampullary	TVA + HGD	I	NCP5	TA + LGD	I
CP6	Duodenum	TVA + HGD	0	NCP6	TA + LGD	II
CP7	Duodenum	VA + HGD	IV	NCP7	TA + LGD	II
CP8	Duodenum	TA + LGD	I	NCP8	TVA + LGD	II
CP9	Duodenum	TA + LGD	IV	NCP9	TA + LGD	III
CP10	Duodenum	TVA + HGD	IV	NCP10	TVA + LGD	IV
CP11	Duodenum	TVA + LGD	IV	NCP11	TA + LGD	III
CP12	Duodenum	TA + LGD	N/A ^b	NCP12	TVA + LGD	III

TA= Tubular, TVA = Tubulovillous, VA= Villous Adenoma, LGD= low-grade, HGD= high-grade dysplasia. ^aOnly had one EGD, in which ampullary cancer with no other duodenal polyposis was diagnosed. ^bNo EGD reports available

Table 2: Clinical and Endoscopic Characteristics of FAP Cases and FAP Controls

		FAP Cases (n=12)	FAP Controls (n=12)	P
Age		48.9 +/- 11.4	49.7 +/- 11.7	0.875
Male Gender		7(58%)	4(33%)	0.414
White		11(92%)	11(92%)	1
Sulindac/Celecoxib use		3(25%)	5(42%)	0.667
Polyp Histology	TA	6 (50%)	7 (58%)	0.68
	TVA	4 (33%)	5 (42%)	
	VA	2 (17%)	0 (0%)	
Polyp Dysplasia	LGD	8 (67%)	12 (100%)	0.093
	HGD	4 (33%)	0 (0%)	
		FAP Cases (n=11)	FAP Controls (n=12)	P
Polyp Number	0 to 5	4(36%)	5(42%)	0.648
	6 to 20	1(9%)	3(25%)	
	>20	6(55%)	4(33%)	
Polyp Size (mm)	0 to 5	2(18%)	4(33%)	0.358
	6 to 10	2(18%)	4(33%)	
	>10	7(64%)	4(33%)	

TA= Tubular, TVA = Tubulovillous, VA= Villous Adenoma, LGD= low-grade, HGD= high-grade dysplasia. One FAP case did not have endoscopy report with polyp number or size available, so analysis was performed on the remaining 11 FAP cases

Table 3: Representative DEGs Grouped by Cellular Function/Pathway

Pathway	DEG	Fold Change			
		Ca-No	Ad-No	Ca-Ad	Ad-Ad
Brush-Border Digestion/Absorption	<i>SI</i>	-5.1	-	-	-2.2
	<i>LCT</i>	-2.5	-2.1	-	-2.8
	<i>TMPRSS15</i>	-4.8	-2.9	-	-
Non-Brush Border Ion Homeostasis	<i>CLCA1</i>	-2.1	-	-2.8	-2.3
	<i>SLC12A2</i>	3.4	3.2	-	-
Lipid Metabolism	<i>APOA4</i>	-5.5	-3.1	-	-3.0
	<i>APOB</i>	-5.7	-3.2	-	-
Carbohydrate Metabolism	<i>ALDOB</i>	-6.7	-3.1	-	-2.0
	<i>PCK1</i>	-2.4	-	-	-
	<i>GBA3</i>	-2.2	-	-	-
Vitamin A Metabolism	<i>ADH1C</i>	-2.6	-	-3.0	-2.4
	<i>ADH4</i>	-2.3	-	-	-2.1
	<i>RBP2</i>	-5.7	-3.3	-	-2.4
Phase I/II Metabolism	<i>CYP2C9</i>	-2.3	-	-	-
	<i>GSTA1</i>	-3.2	-2.3	-	-
	<i>GSTA2</i>	-2.8	-2.1	-	-
	<i>UGT2B17</i>	-2.5	-	-	-
Cell Adhesion/ECM Interactions	<i>COL12A1</i>	4.4	-	3.4	-
	<i>FN1</i>	5.1	-	4.8	-
	<i>SPP1</i>	7.4	-	5.9	-
	<i>MMP1</i>	4.2	2.4	-	-
	<i>MMP7</i>	2.7	-	-	-
	<i>POSTN</i>	4.9	-	4.5	-
	<i>CEACAM5</i>	8.5	4.7	3.2	-
	<i>CEACAM6</i>	6.0	3.5	-	-
Defensins	<i>SULF1</i>	4.3	-	3.7	-
	<i>DEFA5</i>	-3.2	-	-4.6	-
Other	<i>DEFA6</i>	-2.9	-	-3.6	-
	<i>IL8</i>	2.6	-	2.3	-
	<i>CD44</i>	2.1	-	-	-
	<i>ANXA10</i>	-	-	-	-2.1

Negative fold change indicates downregulation and positive fold change indicates upregulation in the more advanced sample. Ca-No= Cancer tissue vs Normal tissue from FAP cases; Ad-No= Adenoma tissue vs Normal tissue from FAP cases; Ca-Ad= Cancer tissue vs Adenoma tissue from FAP cases; Ad-Ad= Adenoma tissue from FAP cases vs Adenoma tissue from FAP controls

Table 4: Transcriptional Changes in Adenoma-Normal Comparison

Neoplastic Change	Cellular Function/Pathway	Expression Change (DEG)	Rationale
Enterocyte de-differentiation to immature crypt phenotype	Brush Border Metabolism	D (<i>LCT, TMPRSS15</i>)	Expression increases in the crypt-villus axis
	Lipid Metabolism	D (<i>APOA4, APOB</i>)	
	Non-BB Metabolism	U (<i>SLC12A2</i>)	Expression decreases in the crypt-villus axis
Warburg Effect^a	Carbohydrate Metabolism	D (<i>ALDOB</i>)	Gluconeogenic enzyme
Decreased production of all-trans-retinoic acid (ATRA)^b	Vitamin A Metabolism	D (<i>RBP2</i>)	Transports dietary vitamin A into enterocytes for conversion to ATRA.
Impaired ROS/carcinogen defense	Phase I/II Metabolism	D (<i>GSTA1/2</i>)	Metabolize carcinogens via glutathione-S-transferase activity and protect cells from ROS via glutathione peroxidase activity

D=Downregulated; U=Upregulated in adenoma tissue vs normal tissue from FAP cases. ^aWarburg Effect refers to tumor cell preference for glycolysis over gluconeogenesis and aerobic respiration. ^bATRA suppresses tumorigenesis in part by blocking COX-2 induction

Table 5: Transcriptional Changes in Cancer-Adenoma Comparison

Neoplastic Change	Cellular Function/Pathway	Expression Change (DEG)	Rationale
Goblet cell de-differentiation	Non-BB Metabolism	D (<i>CLCA1</i>)	Highly and selectively expressed in goblet cells
Paneth cell de-differentiation	Defensin Signaling	D (<i>DEFA5/6</i>)	Exclusively expressed in small intestinal Paneth cells
Decreased production of all-trans-retinoic acid (ATRA)^a	Vitamin A Metabolism	D (<i>ADH1C</i>)	Oxidizes retinol into all-trans retinaldehyde, which is converted into ATRA in enterocytes.
Increased tumor invasiveness	Cell Adhesion/ECM Interactions	U (<i>COL12A1</i>)	Stimulates desmoplastic reaction
		U (<i>FN1, SPP1</i>)	Functions in integrin-mediated cell adhesion
		U (<i>POSTN</i>)	Pro-angiogenesis factors
	Other	U(<i>IL8</i>)	

D=Downregulated; U=Upregulated in cancer tissue vs adenoma tissue from FAP cases. ^aATRA suppresses tumorigenesis in part by blocking COX-2 induction.

Table 6: Gene expression analysis for 4 DEGs from Human Transcriptome Array (HTA) vs confirmatory PCR

Gene	Comparison	HTA array				PCR		
		n	AFC	P	FDR	n	AFC	P
<i>SPP1</i>	Ca-No	12	7.45	<.001	0.02	8	44.84	0.008
	Ad-No	12	<2	NS	NS	8	1.84	0.11
	Ca-Ad	12	5.87	<.001	0.06	10	23.51	0.002
	Ad-Ad	12,12	<2	NS	NS	10,12	1.12	0.39
<i>CEACAM5</i>	Ca-No	12	8.52	<.001	0.02	9	3.94	0.098
	Ad-No	12	4.73	<.001	0.06	9	2.41	0.13
	Ca-Ad	12	3.23	0.027	0.08	10	1.68	0.098
	Ad-Ad	12,12	<2	NS	NS	10,12	2.14	0.97
<i>APOA4</i>	Ca-No	12	-5.48	<0.001	0.02	10	-27.54	0.002
	Ad-No	12	-3.14	0.001	0.07	10	-3.30	0.084
	Ca-Ad	12	-1.64	<0.001	0.06	10	-8.33	0.004
	Ad-Ad	12,12	-3.04	0.017	0.07	10,12	-5.80	0.004
<i>ANXA10</i>	Ca-No	12	<2	NS	NS	9	1.47	0.3
	Ad-No	12	2.26	0.043	0.30	9	5.96	0.012
	Ca-Ad	12	-1.51	0.002	0.07	10	-4.01	0.002
	Ad-Ad	12,12	-2.10	0.012	0.07	10,12	-2.09	0.081

Negative fold change indicates downregulation and positive fold change indicates upregulation in the more advanced sample. Ca-No= Cancer tissue vs Normal tissue from FAP cases; Ad-No= Adenoma tissue vs Normal tissue from FAP cases; Ca-Ad= Cancer tissue vs Adenoma tissue from FAP cases; Ad-Ad= Adenoma tissue from FAP cases vs Adenoma tissue from FAP controls. For Ad-Ad comparison, number of samples in FAP case group and in FAP control group are shown.

Supplemental Table 1: DEGs in Duodenal Neoplasia in FAP

Aim	Comparison	# DEGs	# Protein Coding DEGs		# Non-Protein Coding DEGs
			Downregulated	Upregulated	
Adenoma-Carcinoma Sequence in Cases	Cancer-Normal	169	57	64	48
	Adenoma-Normal	25	14	5	6
	Cancer-Adenoma	26	7	19	0
Cases vs Control Adenomas	Adenoma-Adenoma	18	13	0	5

Supplemental Table 2: List of DEGs in each comparison

Cancer-Normal			Cancer-Adenoma		Adenoma-Normal		Adenoma-Adenoma	
Downregulated	Upregulated		Downregulated	Upregulated	Downregulated	Upregulated	Downregulated	Upregulated
ALDOB	SPTBN1	BGN	DEFA5	ANTXR1	RBP2	SNORD12B	ADH1C	SNORA76
APOB	SNORD3D	LOC100507056	DEFA6	RAB31	APOB	RNU105A	ADH4	
RBP2	GAPDH	SNORD14E	ADH1C	VCAN	APOA4	SNORA68	ALDOB	
APOA4	HIST1H1E	MIR614	CLCA1	MMP2	ALDOB	MMP1	ANXA10	
SI	SOX9	TXN	CPS1	IL8	TMPRSS15	SNORA74A	APOA4	
TMPRSS15	PGK1	REG4	MT1G	BGN	SLC26A3	SNORD78	C17orf78	
SLC26A3	KIF5B	SNORD41	HMGS2	SPARC	GSTA1	SNORD14A	CES1	
GIP	KPNA2	SNORD70		SCD	CYP3A4	SLC12A2	CLCA1	
MEP1B	SNORD3C	SNORA21		COL6A3	MEP1B	CEACAM6	CPS1	
DEFA5	MIR612	SCD		GREM1	SLC15A1	REG4	DMBT1	
GSTA1	CD44	GREM1		CEACAM5	MME	CEACAM5	FABP1	
SLC15A1	ANTXR1	SNORD12C		COL1A1	GSTA2		LCT	
CYP3A4	SNORA65	COL6A3		COL1A2	LCT		LOC399753	
DEFA6	IFITM1	TMSB10		COL12A1	ASAH2C		MIR548O2	
IGHM	SLC2A1	SLC12A2		COL3A1			MIR548X	
GSTA2	ANO1	SNORD60		SULF1			RBP2	
SST	KIAA1199	SNORD18A		POSTN			SI	
ASAH2C	SNORD105B	SNORA74A		FN1				
MME	SNORD1A	SNORD14A		SPP1				
FOLH1	VCAN	COL1A2						
ADH1C	RAB31	COL3A1						
SLC5A1	MIR622	SNORD12B						
FABP2	ODC1	COL1A1						
UGT2A3	SNORA10	SNORD16						
FOLH1B	SNORA76	MMP1						
MTTP	SNORD97	SULF1						
LCT	HOXB5	COL12A1						
UGT2B17	KRT18	SNORD78						
MEP1A	RMRP	SNORD12						
CPS1	SNORA71A	POSTN						
PCK1	TGFB1	FN1						
MS4A10	HIST1H2BI	CEACAM6						
ACE2	TNS4	SNORD37						
AKR1B10	IGHG4	SPP1						
ADH4	RNU12	CEACAM5						
CYP2C19	IFITM2	SNORD83A						

REG3A	THBS2	IL8			
ASAH2	YWHAZP3	IFI27			
ADAMDEC1	SNORA52	MMP14			
CYP2C9	MIR3687	MMP12			
MGAM	PLP2	SNORD92			
PDK4	IFITM3	RNU6ATAC			
C19orf77	TNC	SPARC			
CREB3L3	HIST1H2BK	MMP7			
GBA3	SNORA68	ANXA2P2			
ANPEP	MXRA5	SNORA71C			
DMBT1	MMP2	SNORD14B			
ITLN2	HSP90AB1				
MT1H	GDF15				
LOC100653084	RPL19				
SLC4A4	PKM				
LOC100134256	DUOX2				
CYP2C18	SNORD15A				
CLCA1	THBS1				
SLC2A2	SNORA31				
LOC100507600	SNORD126				
HSD17B2	TUBA1B				
ALDH1A1	MYOF				
SLC28A2	RNU105A				
SLC6A19	ANXA2				
DHRS11	SNORD99				

Supplemental Table 3: FAP cases with undefined results on PCR

DEG	Normal	Adenoma	Cancer	Adenoma(control)
SPP1	5,9,10,11	10, 11	10	None
CEACAM5	5,10,11	10, 11	10	None
APOA4	10,11	10,11	10	None
ANXA10	10,11,12	10,11	10	None
SI	10,11	6,9,10,11	3,4,6,8,9,10	None

FAP case numbers for patients with undefined results on PCR verification, grouped by DEG. No FAP control had any undefined results

**Supplemental Table 4:
Sample year and fixative for
each FAP case**

FAP Case	Year	Fixative
1	2003	FFPE
2	2003	FFPE
3	2003	FFPE
4	2004	FFPE
5	2007	FFPE
6	2008	FFPE
7	2008	FFPE
8	2009	FFPE
9	2013	FFPE
10	2000	Hollande's
11	1999	Hollande's
12	1991	Hollande's

FFPE= formalin-fixed paraffin-embedded sample

