High expression of insulinoma-associated protein 1 (INSM1) distinguishes colorectal mixed and pure neuroendocrine carcinomas from conventional adenocarcinomas with diffuse expression of synaptophysin

Supplementary Tables S1–S8

Table S1. Correlation between INSM1 expression and expression of chromogranin A in conventional adenocarcinomas

	INSM1 negative (IRS 0-1)	INSM1 weak (IRS 2-3)	INSM1 moderate (IRS 4-8)	Total number	*p* value
Conventional adenocarcinoma chromogranin A negative (IRS 0-1)	816	92	9	*917*	
Conventional adenocarcinoma chromogranin A weak (IRS 2-3)	68	36	2	*106*	
Conventional adenocarcinoma chromogranin A moderate (IRS 4-8)	4	5	1	*10*	
Total number	*888*	*133*	*12*	*1033*	*<0.001*

Table S2. Correlation between INSM1 expression and expression of chromogranin A in MANEC/NEC

	INSM1 weak (IRS 2-3)	INSM1 moderate (IRS 4-8)	INSM1 strong (IRS 9-12)	Total number	*p* value
NEC/MANEC chromogranin A negative (IRS 0-1)	1	4	3	*8*	
NEC/MANEC chromogranin A weak (IRS 2-3)	1	1	4	*6*	
NEC/MANEC chromogranin A moderate (IRS 4-8)	0	1	1	*2*	
NEC/MANEC chromogranin A strong (IRS 9-12)	0	1	4	*5*	
Total number	*2*	*7*	*12*	*21*	*0.707*

Table S3. Survival associations of INSM1 expression groups among conventional adenocarcinomas including mean survival times

	Overall *n* (%)	Mean overall survival (SE) [months]	*p* value	Mean disease specific survival (SE) [months]	*p* value	Mean disease free survival (SE) [months]	*p* value
INSM1 expression groups			*0.849*		*0.273*		*0.884*
Conventional Adenocarcinoma INSM1 negative (IRS 0-1)	*888 (86.0%)*	79.04 (1.7)		87.08 (1.7)		82.71 (1.8)	
Conventional Adenocarcinoma INSM1 weak (IRS 2-3)	*133 (12.9%)*	81.98 (4.3)		94.47 (4.2)		83.95 (4.7)	
Conventional Adenocarcinoma INSM1 moderate (IRS 4-8)	*12 (1.2%)*	80.93 (15.5)		89.01 (15.3)		74.49 (17.4)	

Table S4. Survival associations of INSM1 expression groups among conventional adenocarcinomas compared to colorectal MANEC/NEC including mean survival times

	Overall n (%)	Mean overall survival (SE) [months]	*p* value	Mean disease specific survival (SE) [months]	*p* value	Mean disease free survival (SE) [months]	*p* value
INSM1 expression			*<0.001*		*<0.001*		*<0.001*
Conventional Adenocarcinoma INSM1 negative (IRS 0-1)	*888 (84.3%)*	79.04 (1.7)		87.08 (1.7)		82.71 (1.8)	
Conventional Adenocarcinoma INSM1 weak (IRS 2-3)	*133 (12.6%)*	81.98 (4.3)		94.47 (4.2)		83.95 (4.7)	
Conventional Adenocarcinoma INSM1 moderate (IRS 4-8)	*12 (1.1%)*	80.93 (15.5)		89.01 (15.3)		74.49 (17.4)	
MANEC/NEC	21 *(2.1%)*	26.51 (6.2)		26.51 (6.2)		22.99 (6.1)	

Table S5. Multivariate analysis for overall survival including INSM1 expression groups in conventional adenocarcinomas compared to MANECs including UICC stage, WHO-grade, microsatellite status, resection status, age, and gender

		HR (OS)	Lower CI (95%)	Upper CI (95%)	*p* value
INSM1 expression groups in conventional adenocarcinoma vs MANEC/NEC					*0.014*
	Conventional adenocarcinoma INSM1 negative (lRS 0-1)	1			
	Conventional adenocarcinoma INSM1 low (IRS 2-3)	0.978	0.727	1.316	
	Conventional adenocarcinoma INSM1 moderate (IRS 4-8)	0.625	0.232	1.685	
	MANEC/NEC	2.167	1.167	4.023	
UICC Stage					*<0.001*
	I	1			
	II	1.481	1.068	2.052	
	III	1.653	1.194	2.288	
	IV	4.198	2.931	6.014	
Resection status					*<0.001*
	R0	1			
	R1	1.968	1.352	2.864	
	R2	1.975	1.279	3.050	
Microsatellite status					*0.060*
	Microsatellite stable	1			
	Microsatellite unstable	0.737	0.536	1.013	
WHO Grade					*0.002*
	Low grade (former G1/G2)	1			
	High grade (former G3)	1.398	1.134	1.724	
Gender					*0.022*
	Male	1			
	Female	0.790	0.645	0.967	
Age					*<0.001*
	Below median	1			
	Median and above	1.852	1.520	2.257	

Table S6. Multivariate analysis for disease-free survival including INSM1 expression groups in conventional adenocarcinomas compared to MANECs including UICC stage, WHO-grade, microsatellite status, resection status, age, and gender

		HR (DFS)	Lower CI (95%)	Upper CI (95%)	p-value
INSM1 expression groups in conventional adenocarcinoma vs MANEC/NEC					*0.030*
	Conventional adenocarcinoma INSM1 negative (IRS 0-1)	1			
	Conventional adenocarcinoma INSM1 low (IRS 2-3)	1.014	0.726	1.416	
	Conventional adenocarcinoma INSM1 moderate (IRS 4-8)	0.782	0.288	2.127	
	MANEC/NEC	1.936	1.066	3.514	
UICC Stage					*<0.001*
	I	1			
	II	1.985	1.245	3.166	
	III	3.808	2.449	5.922	
	IV	8.618	5.375	13.818	
Resection status					*<0.001*
	R0	1			
	R1	2.183	1.496	3.185	
	R2	1.567	1.000	2.456	
Microsatellite status					*0.010*
	Microsatellite stable	1			
	Microsatellite unstable	0.595	0.401	0.883	
WHO Grade					*0.008*
	Low grade (former G1/G2)	1			
	High grade (former G3)	1.369	1.086	1.725	
Gender					*0.577*
	Male	1			
	Female	0.939	0.753	1.171	
Age					*0.097*
	Below median	1			
	Median and above	1.202	0.968	1.494	

Table S7. Cohort characteristics and expression of synaptophysin, chromogranin A, and INSM1 in the conventional CRC validation cohort

	Overall *n* (%)
Age	
Below median	*135 (49%)*
Median and above	*139 (51%)*
Sex	
Male	*153 (57%)*
Female	*121 (43%)*
Specimen type	
Resection	*274 (100%)*
Biopsy	*0 (0%)*
Expression of INSM1	
Negative (IRS 0-1)	*254 (93%)*
Weak (IRS 2-3)	*20 (7%)*
Moderate (IRS 4-8)	*0 (0%)*
Strong (IRS 9-12)	*0 (0%)*
Expression of chromogranin A	
Negative (IRS 0-1)	*247 (90%)*
Weak (IRS 2-3)	*27 (10%)*
Moderate (IRS 4-8)	*0 (0%)*
Strong (IRS 9-12)	*0 (0%)*
Expression of synaptophysin	
Negative	*236 (86%)*
Low (1-9%)	*23 (8%)*
Moderate (10-29%)	*10 (4%)*
High (30-100%)	*5 (2%)*
pT	
1	*14 (5%)*
2	*50 (18%)*
3	*169 (62%)*
4	*40 (15%)*
pN	
0	*145 (53%)*
1	*71 (25%)*
2	*58 (21%)*
pM	
0	*215 (79%)*
1	59 (21%)
UICC Stage	
I	*51 (19%)*
II	*81 (29%)*
III	*82 (30%)*
IV	*60 (22%)*
WHO grade	
Low-grade	*204 (75%)*
High-grade	*70 (25%)*
Resection margin	
R0	*265 (97%)*
R1	*9 (3%)*

Table S8. Cohort characteristics and expression of synaptophysin, chromogranin A, and INSM1 in the MANEC/NEC validation cohort

	Overall *n* (%)
Age	
Below median	8 (42%)
Median and above	11 (58%)
Sex	
Male	10 (53%)
Female	9 (47%)
Specimen type	
Resection	11 (58%)
Biopsy	8 (42%)
Tumor type	
MANEC	4 (21%)
Large Cell NEC	11 (58%)
Small Cell NEC	4 (21%)
Expression of INSM1	
Negative (IRS 0-1)	0 (0%)
Weak (IRS 2-3)	4 (21%)
Moderate (IRS4-8)	6 (32%)
Strong (IRS 9-12)	9 (47%)
Expression of chromogranin A	
Negative (IRS 0-1)	12 (63%)
Weak (IRS 2-3)	1 (5%)
Moderate (IRS4-8)	5 (27%)
Strong (IRS 9-12)	1 (5%)
Expression of synaptophysin	
Negative	0 (0%)
Focal	1 (6%)
Diffuse	18 (94%)
pT	
Not applicable (biopsies)	11 (58%)
1	0 (0%)
2	2 (11%)
3	6 (31%)
4	0
pN	
Not applicable (biopsies)	11 (58%)
0	1 (5%)
1	2 (11%)
2	5 (26%)
pM	
Not applicable (biopsies)	11 (58%)
0	7 (37%)
1	1 (5%)
UICC Stage	
Not applicable (biopsies)	11 (58%)
1	1 (5%)
2	0 (0%)

3	6 (32%)	
4	1 (5%)	
WHO grade		
Low-grade	0	
High-grade	19 (100%)	
Resection margin		
Not applicable (biopsies)	11 (58%)	
R0	7 (37%)	
R1	1 (5%)	