of some steps during EMT [7,8]. Previous studies have proved that H3K9 methyltransferase G9a is involved in the epigenetic control of phenotypic and cellular plasticity during EMT [6]. In different tumour types, the molecular relation between H3K9 methylations and cancer progression is still an important point of research, the clinical significance and functional role of H3K9 methyltransferase G9a in colon cancer is still an area of controversy [9].

Sprouty (SPRY) is an intracellular regulator of receptor tyrosine kinase signalling involved in growth, differentiation and tumour genesis. Four family members of SPRY (SPRY1–4) have been identified [10,11]. Sprouty proteins have been implicated in the regulation of the biological processes central to tumour growth, development and metastasis, including cell proliferation, migration, invasion and survival [12]. Up- or down-regulation of Sprouty 1 in a variety of neoplasms consider it as a possible tumour marker and a promising target for drug intervention [13].

Therefore, our study aimed to evaluate the significance of H3K9 methyltransferase G9a and Sprouty 1expression in colorectal carcinoma (CRC), detecting their prognosis role in patients with CRC.

Patients and Methods

Immunohistochemical expressions of H3K9 methyltransferase G9a and Sprouty 1 were evaluated in sections from 50 paraffin blocks retrieved from 50 cases of colorectal carcinoma (CRC) that were collected from archives of Pathology and Oncology Departments, Faculty of Medicine, Zagazig, Beni-Suif Universities and National Cancer Institute, Cairo university during the period from April 2013 to April 2016. The seventh edition of the American Joint Committee on Cancer staging system (AJCC-7) classification and the World Health Organization (WHO) classification were used for pathologic staging and a pathologic grading respectively [14,15].

Clinical criteria as sex, age, tumour size, grade and stage have been identified by retrospective examination of the slides and the patient's files.

Approval for the study was obtained from the ethics committee of the contributing centres. Patients with stages I-III undergone surgery and adjuvant chemotherapy for stage III and high risk stage II, while chemotherapy was the first line for metastatic CRC. None of the patients had received preoperative chemotherapy or radiation therapy.

Immunohistochemical staining

Formalin-fixed, paraffin-embedded tissue sec¬tions (5 μm-thicks) were deparaffinized with xylene and rehydrated. For antigen retrieval, sec-tions were placed in either 10 mM Tris base, 1 mM EDTA solution at pH 9.0 10 mM sodium citrate buffer at pH 6.0 and exposed to repeated (twice) microwave heating of 10 min at 750W. After 10 min incubation with 3% hydrogen peroxide for inactivation of endogenous peroxidase activity, sections were blocked with DAKO blocking buffer followed by incubation at 4°C overnight with primary anti Sprouty1 mouse monoclonal antibody (1:300) (ab56670 Abcam, Cambridge, MA, USA) and rabbit monoclonal anti-G9a antibody (1:500 dilution; ab185050, Abcam, Cambridge, MA, USA). Sections of heart tissue and lymph node were used as positive controls for Sprouty1 and G9a respectively. Specimens were then incubated with secondary antibody using EnVision Plus kit (DAKO) for 30 min and then with diaminobenzidine chromogen for 5 min. All slides were counterstained with hematoxylin. For negative controls, the primary antibodies were omitted and replaced with normal saline. Under light microscope, staining of the epithelial cells was evaluated and scored by three pathologists. Representative slides were photographed.

Determination of Sprouty 1 Expression by Immunohistochemical Assay

The positive Sprouty 1 expression cells have brownish-yellow granules in their cytoplasm. Semi-quantitative scoring based on the intensity and the percentage of immunoreactive cells. A four-value intensity score (0, no immunoreactivity; 1, weak; 2, moderate and 3, strong) was used as well as a four-value extent score as follows; (0, none; 1, 1-33%; 2, 34-66%; and 3, 67-100%) [16], then the average intensity of the stain and extent of stained tumor cells scores were multiplied resulting in a 9-point score ranging from 0 (no staining) to 9 (extensive, strong staining) for each case.

We consider the cut off value 4 above which is considered high total score and below which low total score.

Determination of H3K9 methyltransferase G9a expression by immunohistochemical assay

The positive H3K9 methyltransferase G9a expression cells exhibited to have brownish granules in their nuclei. Its expression was graded on the basis of the following standards: cancer cells that were unstained or that were stained less than10% classified as negative and cancer cells that were stained more than10% identified as positive [17].

The intensity score (0, no immunoreactivity; 1, weak; 2, strong) was used as well as the extent score (1, 1-10%; 2, 10-50%; and 3, 50-100%). The average intensity of the stain and extent of stained tumor cells were then multiplied yielding a 6-point score ranging from 0 (no staining) to 6 (extensive, strong staining) for each case.

High expression was defined as either positive staining present in 10%–50% of the cells (staining intensity score=2) or more than 50% of the cells (staining intensity score=3) [18].

Statistics Analysis

Continuous variables were expressed as the mean ± SD and median (range), and the categorical variables were expressed as a number (percentage). Mann Whitney U test was used to compare between two groups of non-normally distributed variables. Relapse Free Survival (RFS) was calculated as the time from end of treatment to date at which recurrence was detected. Stratification of OS and RFS was done according to all clinic-pathological features and immune-histochemical markers. These time-to-death distributions were estimated using the method of Kaplan-Meier plot. A p-value <0.05 was considered significant. All statistics were performed using SPSS 22.0 for windows (SPSS Inc., Chicago, IL, USA) and MedCalc windows (MedCalc Software bvba 13, Ostend, Belgium).

Results

Patients characteristics

The clinical characteristics of the 50 patients with colorectal carcinoma (CRC) that were included in the study are summarized in Table 1.

30 (60%) males and 20 (40%) females with age ranged from (29-80) years (Mean: 58.12 ± 12.02 years), 45 (90%) cases were conventional adenocarcinoma.

Characteristics	All (N=50) No. (%)		SPRY1				p-value	H3K9 methyl-transferase G9a				p-value
			Low (N=23) No. (%)		High (N=27) No. (%)			Low (N=22) No. (%)		High (N=28) No. (%)		
Age (years)												
Mean ± SD	58.12	±12.02	58.52	±13.45	57.78	±10.91	0.830*	55.36	±10.03	60.29	±13.15	0.153*
Median (Range)	57	(29-80)	60	(29-80)	55	(30-75)		55	(30-75)	62	(29-80)	
<60 years	26	-52%	11	-47.80%	15	-55.60%	0.586‡	14	-63.60%	12	-42.90%	0.144‡
≥60 years	24	-48%	12	-52.20%	12	-44.40%		8	-36.40%	16	-57.10%	
Sex												
Male	30	-60%	16	-69.60%	14	-51.90%	0.203‡	12	-54.50%	18	-64.30%	0.485‡
Female	20	-40%	7	-30.40%	13	-48.10%		10	-45.50%	10	-35.70%	
Location												
Ascending colon	15	-30%	9	-39.10%	6	-22.20%	0.265‡	1	-4.50%	14	-50%	0.002‡
Transverse colon	4	-8%	3	-13%	1	-3.70%		1	-4.50%	3	-10.70%	
Descending colon	5	-10%	2	-8.70%	3	-11.10%		3	-13.60%	2	-7.10%	
Rectosigmoid	26	-52%	9	-39.10%	17	-63%		17	-77.30%	9	-32.10%	
Size (cm)												
≤ 5 cm	22	-44%	4	-17.40%	18	-66.70%	<0.001‡	18	-81.80%	4	-14.30%	<0.001‡
> 5 cm	28	-56%	19	-82.60%	9	-33.30%		4	-18.20%	24	-85.70%	
Pathological Type												
Adenocarcinoma	45	-90%	19	-82.60%	26	-96.30%	0.167‡	22	-100%	23	-82.10%	0.059‡
Mucinous	5	-10%	4	-17.40%	1	-3.70%		0	0%	5	-17.90%	
Grade												
Grade I	7	-14%	2	-8.70%	5	-18.50%	0.069·	7	-31.80%	0	0%	<0.001·
Grade II	35	-70%	15	-65.20%	20	-74.10%		15	-68.20%	20	-71.40%	
Grade III	8	-16%	6	-26.10%	2	-7.40%		0	0%	8	-28.60%	
Lymph node												
Negative	21	-42%	4	-17.40%	17	-63%	0.001‡	17	-77.30%	4	-14.30%	<0.001‡
Positive	29	-58%	19	-82.60%	10	-37%		5	-22.70%	24	-85.70%	
DM												
Absent	40	-80%	15	-65.20%	25	-92.60%	0.030‡	22	-100%	18	-64.30%	0.001‡
Present	10	-20%	8	-34.80%	2	-7.40%		0	0%	10	-35.70%	
Stage												
Stage I	8	-16%	2	-8.70%	6	-22.20%	0.002·	8	-36.40%	0	0%	<0.001·
Stage II	13	-26%	2	-8.70%	11	-40.70%		9	-40.90%	4	-14.30%	
Stage III	19	-38%	11	-47.80%	8	-29.60%		5	-22.70%	14	-50%	
Stage IV	10	-20%	8	-34.80%	2	-7.40%		0	0%	10	-35.70%	
SPRY1												
Low	23	-46%						2	-9.10%	21	-75%	<0.001‡
High	27	-54%						20	-90.90%	7	-25%	
H3K9 methyl-transferase G9a												
Low	22	-44%	2	-8.70%	20	-74.10%	<0.001‡					
High	28	-56%	21	-91.30%	7	-25.90%						

Categorical variables were expressed as number (percentage), continuous variables were expressed as mean ± SD and median (range)
*Independent samples Student's t-test; ‡Chi-square test; ·Chi-square test for trend; p<0.05 is significant.

Table 1: correlation between clinicopathological features, SPRY1 and H3K9 methyl-transferase G9a expression in colorectal carcinoma.

H3K9 methyltransferase G9a immunoexpression and its correlation clinicopathological features of CRC patients

Of the 50 patients, G9a had nuclear expression in 56% (28/50) patients. The high expression of G9a in CRC was significantly correlated with the tumor size, grade, stage, presence of lymph node, distant metastases (p<0.001 for all), and tumor site (p=0.002), No significant correlation was found between G9a expression and age or sex of our patients (Table 1 and Figure 1).

Sprouty 1 immunoexpression and their correlation clinicopathological features of the patients

High cytoplasmic expression of Sprouty 1 was detected in 27 out of 50 (54%) cases of CRC.

The high expression of SPRY1 was negatively correlated with the tumour size, presence of lymph node metastases (p<0.001 for both), stage (p=0.002), and presence of distant metastases (p=0.03). No significant correlation was found between SPRY1 expression, age, sex, grade, and size or tumour site (Table 1 and Figure 2).

Survival analysis

After a median follow up of 32 (range; 8-35) months, seventeen cases had recurrence of the tumor after treatment, 16 of them had high H3K9 methyltransferase G9a expression and 14 of them had low sprouty 1 expression.

The 2-year recurrence free survival rate of our patients was 95.5% and 16.7% in patients with low and high methyltransferase G9a

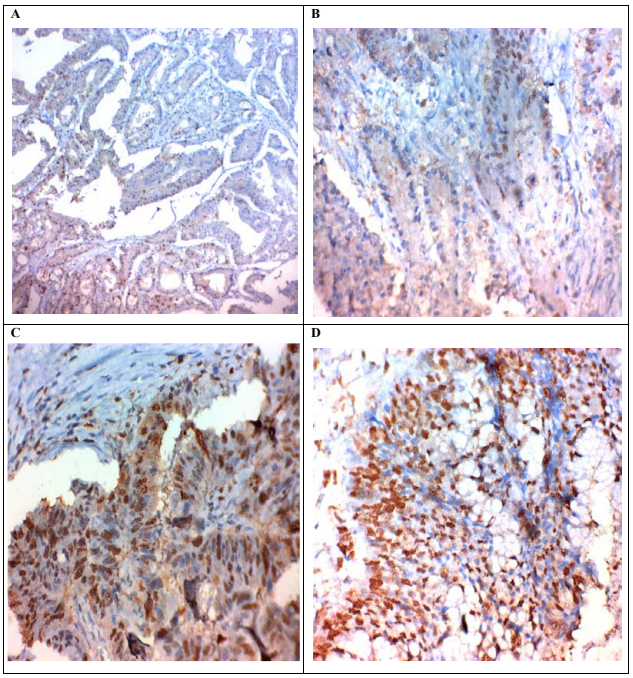

Figure 1: Immunohistochemical staining of H3K9 methyltransferase G9a in CRC: (A) Low Immunohistochemical expression in the nucleus of CRC grade I, stage Ix200; (B) Low Immunohistochemical expression in the nucleus of CRC grade II, stage II x400. (C) High Immunohistochemical expression in the nucleus of CRC grade grade III, stage IVx400. (D) High Immunohistochemical expression in the nucleus of mucinous CRC grade III, stage III x400.Note: High H3K9 methyltransferase G9a immunohistochemical expressionin in high grade and stage CRC and low expression in low grade and stage CRC Magnification: A the original magnification was ×200, B, C and D, the original magnification was ×400.

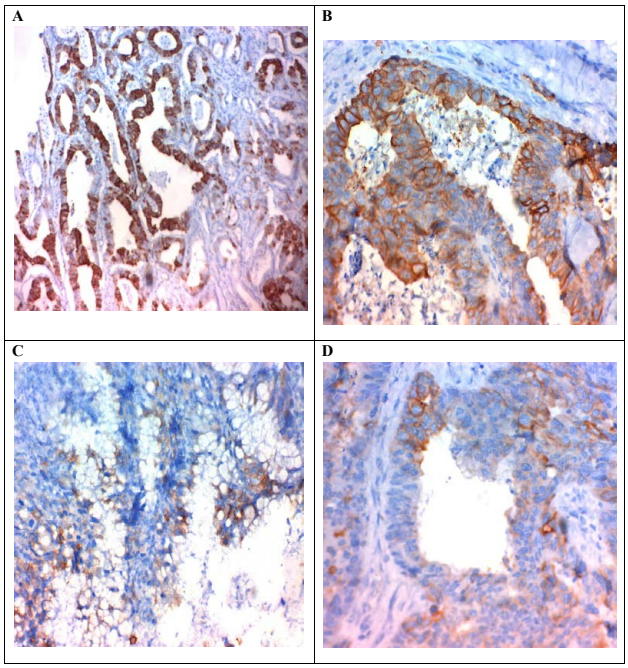

Figure 2: Immunohistochemical staining of Sprouty 1 in CRC (A) High Immunohistochemical expression in the cytoplasm of CRC grade I stage Ix200 (B) High Immunohistochemical expression in the cytoplasm of CRC grade II, stage IIBx400. (C) Low Immunohistochemical expression in the cytoplasm of mucinous CRC grade II, stage IIBx400. (D) Low Immunohistochemical expression in the cytoplasm of CRC grade II, stage IIIx400. Note: Low Sprouty 1 immunohistochemical expression in high grade and stage CRC and High expression in low grade and stage CRC: A the original magnification was ×200, B, C and D, the original magnification was ×400.

expression respectively, and 13.3% and 88% in patients with low and high SPRY1 1expression respectively (p<0.001) (Table 2).

The 2-year overall survival (OS) rate was 38.3% in patients with low SPRY1 expression (p=0.008), and 49.5% in patients with high methyltransferase G9a (p<0.001).

High H3K9 methyltransferase G9a expression was significantly positively correlated with high incidence of recurrence of the cancer, worse recurrence free survival (RFS) rate and worse 2-year overall survival (OS) rate (p<0.001).

SPRY1 expression was significantly negatively correlated with high incidence of recurrence of the cancer, worse recurrence free survival (RFS) rate and worse 2-year overall survival (OS) rate (p<0.001).

Regarding expecting recurrence of CRC in our patients; the sensitivity of only high H3K9 methyltransferase G9a expression was 82.4%, sensitivity of only low SPRY1 expression while the sensitivity of combination of high H3K9 methyltransferase G9a expression and low sprouty 1 expression was 100% and the specificity was 87%.

Cases of colorectal cancer with unfavourable prognosis had low expression of SPRY1 and high expression G9a.

Both markers were negatively correlated to each other (p<0.001; Tables 1-3, Figures 3 and 4).

Discussion

Here, we observed that the expression of H3K9 methyltransferase G9a in CRC was significantly positively correlated with size of the tumour, grade, stage, presence of lymph node and distant metastases (p<0.001), and location of the tumour within the colon (p=0.002) but there was none significant correlation between its expression and age or sex of our patients.

Also, patients with high H3K9 methyltransferase G9a expression had significantly poorer 2-year OS rate (p<0.001) and poorer 2-year local recurrence free survival rate (p<0.001).

So, H3K9 methyltransferase G9a found to be a recent marker for prognosis of CRC, plays an important role in its progression and metastasis and may be a novel therapeutic target for such type of cancer. These results were similar to previous reports in the CRC, serous ovarian carcinoma and endometrial cancer progression and poor prognosis [18-20].

The epigenetic changes and histone modification are important for occurrence and progression of cancer [21,22], H3K9 methyltransferase G9a plays an important role in such epigenetic changes as DNA methylation of embryonic and germ-line genes [23], necessary for the maintenance of the DNA methylation profile of mammalian cells [24], and its deletion in prostate cancer cells inhibited cell growth and led to senescence of malignant cells with telomere abnormalities [25], but the molecular link between H3K9 methylation and cancer progression remains uncertain, although there is a clear correlation among the global H3K9 methylation patterns, clinical outcome, tumour phenotypes and prognostic factors, in cancers of many organs [9]. H3K9 methyltransferase G9a can cooperate with other transcription factors to regulate gene expression [26], and is involved with important

Characteristics	All (N=40) No. (%)		Recurrence				p-value	Recurrence Free Survival (RFS)		
			No (N=23) No. (%)		Yes (N=17) No. (%)			Median RFS (months)		2 years RFS (%)
										p-value§
Age (years)										
Mean ± SD	55.78	±11.81	55.3	±9.81	56.41	±14.39	0.774˙			
Median (Range)	55	(29-80)	55	(30-75)	55	(29-80)				
<60 years	25	-62.50%	15	-60%	10	-40%	0.680‡	NR	60%	0.703
≥60 years	15	-37.50%	8	-53.30%	7	-46.70%		NR	60%	
Sex										
Male	24	-60%	13	-54.20%	11	-45.80%	0.601‡	NR	58.30%	0.831
Female	16	-40%	10	-62.50%	6	-37.50%		NR	62.50%	
Location										
Ascending colon	9	-22.50%	1	-11.10%	8	(88.9%	0.007‡	14 months	11.10%	0.001
Transverse colon	3	-7.50%	1	-33.30%	2	-66.70%		NR	82.60%	
Descending colon	5	-12.50%	4	-80%	1	-20%		NR	100%	
Rectosigmoid	23	-57.50%	17	-73.90%	6	-26.10%		15 months	33.30%	
Size (cm)										
≤ 5 cm	22	-55%	19	-86.40%	3	-13.60%	<0.001‡	NR	90.90%	<0.001
> 5 cm	18	-45%	4	-22.20%	14	-77.80%		13.5 months	22.20%	
Pathological Type										
Adenocarcinoma	40	-100%	23	-57.50%	17	-42.50%	----	NR	60%	----
Mucinous	---	---	---	---	---	---		----	----	
Grade										
Grade I	7	-17.50%	6	-85.70%	1	-14.30%	0.205‡	NR	85.70%	0.101
Grade II	33	-82.50%	17	-51.50%	16	-48.50%		NR	54.50%	
Grade III	---	---	---	---	---	---		----	----	
Lymph node										
Negative	21	-52.50%	18	-85.70%	3	-14.30%	<0.001‡	NR	90.50%	<0.001
Positive	19	-47.50%	5	-26.30%	14	-73.70%		14 months	26.30%	
Stage										
Stage I	8	-50%	7	-87.50%	1	-12.50%	0.001˙	NR	87.50%	<0.001
Stage II	13	-32.50%	11	-84.60%	2	-15.40%		NR	92.30%	
Stage III	19	-47.50%	5	-26.30%	14	-73.70%		14 months	26.30%	
Stage IV	---	---	---	---	---	---		----	----	
SPRY1										
Low	15	-37.50%	1	-6.70%	14	-93.30%	<0.001‡	14 months	13.30%	<0.001
High	25	-62.50%	22	-88%	3	-12%		NR	88%	
H3K9 methyl-transferase G9a										
Low	22	-55%	21	-95.50%	1	-4.50%	<0.001‡	NR	95.50%	<0.001
High	18	-45%	2	-11.10%	16	-88.90%		13.5 months	16.70%	
SPRY1/H3K9 MT G9a										
Low/Low	2	-5%	1	-50%	1	-50%	<0.001‡	NR	50%	<0.001
Low/High	13	-32.50%	0	0%	13	-100%		12 months	76.90%	
High/Low	20	-50%	20	-100%	0	0%		NR	100%	
High/High	5	-12.50%	2	-40%	3	-60%		20 months	40%	

Categorical variables were expressed as number (percentage), continuous variables were expressed as mean ± SD and median (range)
*Independent samples Student's t-test; ‡Chi-square test; ˙Chi-square test for trend; NR denote not reached yet; §Log rank test; p<0.05 is significant; Hazard ratio (95%CI): - SPRY1: 13.157 (4.421 – 38.461); H3K9 methyl-transferase G9a: 36.03 (12.861 – 100.943).

Table 2: correlation between clinicopathological features, SPRY1 and H3K9 methyl-transferase G9a expression and recurrence of colorectal carcinoma.

cancer sustaining cellular activities such as proliferation, autophagy, EMT, metabolic changes, specific responses to hypoxia and cancer stemness [6-8,25]. Previous researches demonstrated that H3K9 methyltransferase G9a is essential for Snail-mediated EMT [6], and required for progression and spread of cancer cells.

The epigenetic changes, unlike genetic mutations, can be reversed to their parent non mutated state by epigenetic therapy [27], which includes anticancer drugs targeting histone deacetylases and histone methyltransferase [28]. The analysis of the mechanisms underlying the roles of epigenetic alteration in tumour progression is still an early stage and the functional roles of H3K9 methyltransferase G9a in cancer remain obscure. However, based on our results data, inhibition of H3K9 methylation is a rational target for cancer therapy.

We found that expression of Spourty 1 was inversely correlated with aggressive clinic pathological features, including size of the tumour and presence of lymph node metastases (p<0.001), stage (p=0.002), and presence of distant metastases (p=0.03). No significant correlation was found between Spourty 1 expression, age or sex of our patients, grade, size and location of the tumour, also we found that Spourty 1 low-expressing patients had significantly poorer 2-year OS

Characteristics	All (N=50) No. (%)	Survival				p-value	Overall Survival (OS)		p-value§
		Alive (N=36) No. (%)		Died (N=14) No. (%)			Median OS (months)	2 years OS (%)	
Age (years)									
Mean ± SD	±12.02	57.78	±10.26	59	±16.12	0.795*			
Median (Range)	(29-80)	55	(30-75)	62	(29-80)				
<60 years	-52%	21	-80.80%	5	-19.20%	0.151‡	NR	80.80%	0.119
≥60 years	-48%	15	-62.50%	9	-37.50%		NR	62.20%	
Sex									
Male	-60%	19	-63.30%	11	-36.70%	0.095‡	NR	63.30%	0.094
Female	-40%	17	-85%	3	-15%		NR	85%	
Location									
Ascending colon	-30%	8	-53.30%	7	-46.70%	0.090‡	NR	51.90%	0.111
Transverse colon	-8%	2	-50%	2	-50%		NR	80.80%	
Descending colon	-10%	5	-100%	0	0%		NR	100%	
Rectosigmoid	-52%	21	-80.80%	5	-19.20%		NR	50%	
Size (cm)									
≤ 5 cm	-44%	22	-100%	0	0%	<0.001‡	NR	100%	<0.001
> 5 cm	-56%	14	-50%	14	-50%		18 months	49.50%	
Pathological Type									
Adenocarcinoma	-90%	34	-75.60%	11	-24.40%	0.126‡	NR	75.60%	0.007
Mucinous	-10%	2	-40%	3	-60%		8 months	40%	
Grade									
Grade I	-14%	7	-100%	0	0%	0.007*	NR	100%	0.004
Grade II	-70%	26	-74.30%	9	-25.70%		NR	74.30%	
Grade III	-16%	3	-37.50%	5	-62.50%		10 months	37.30%	
Lymph node									
Negative	-42%	21	-100%	0	0%	<0.001‡	NR	100%	<0.001
Positive	-58%	15	-51.70%	14	-48.30%		NR	51.30%	
DM									
Absent	-80%	33	-82.50%	7	-17.50%	0.003‡	NR	82.50%	<0.001
Present	-20%	3	-30%	7	-70%		10 months	30%	
Stage									
Stage I	-16%	8	-100%	0	0%	<0.001*	NR	100%	<0.001
Stage II	-26%	13	-100%	0	0%		NR	100%	
Stage III	-38%	12	-63.20%	7	-36.80%		NR	63.20%	
Stage IV	-20%	3	-30%	7	-70%		10 months	30%	
SPRY1									
Low	-46%	9	-39.10%	14	-60.90%	<0.001‡	13 months	38.30%	<0.001
High	-54%	27	-100%	0	-100%		NR	100%	
H3K9 methyl-transferase G9a									
Low	-44%	22	-100%	0	0%	<0.001‡	NR	100%	<0.001
High	-56%	14	-50%	14	-50%		18 months	49.50%	
SPRY1/H3K9 MT G9a									
Low/Low		2	-100%	0	0%	<0.001‡	NR	100%	<0.001
Low/High		7	-33.30%	14	-66.70%		13 months	32.10%	
High/Low		20	-100%	0	0%		NR	100%	
High/High		7	-100%	0	0%		NR	100%	

Categorical variables were expressed as number (percentage), continuous variables were expressed as mean ± SD and median (range)
*Independent samples Student's t-test; ‡Chi-square test; *Chi-square test for trend; NR denote not reached yet; §Log rank test; p<0.05 is significant.

Table 3: correlation between clinicopathological features, SPRY1 and H3K9 methyl-transferase G9a expression and survival of patients with colorectal carcinoma.

(p<0.001) and 2-year local recurrence free survival (p<0.001) than those with high expression of Spourty 1. There is an inverse correlation between the expression of Spourty 1 protein and growth, proliferation, migration and invasion of colorectal cancer cells, also our results identified Spourty 1 as an independent predictor of overall survival and recurrence in CRC. Many previous studies found results that were similar to ours in epithelial ovarian carcinoma (EOC), medullary thyroid carcinoma, breast cancer and osteosarcoma cells that decreased expression of Spourty 1 is a marker of poor prognosis and increasing aggressiveness of that types of cancer [29-31].

The expression of Spourty 1 in human CRC has been explored extensively but contradictory results to that of ours have been reached, Zhang et al. [32] demonstrated that Spourty suppression alters cancer cell morphology from mesenchymal to epithelial type and inhibits spread of cancer with increase in E-cadherin expression and that over

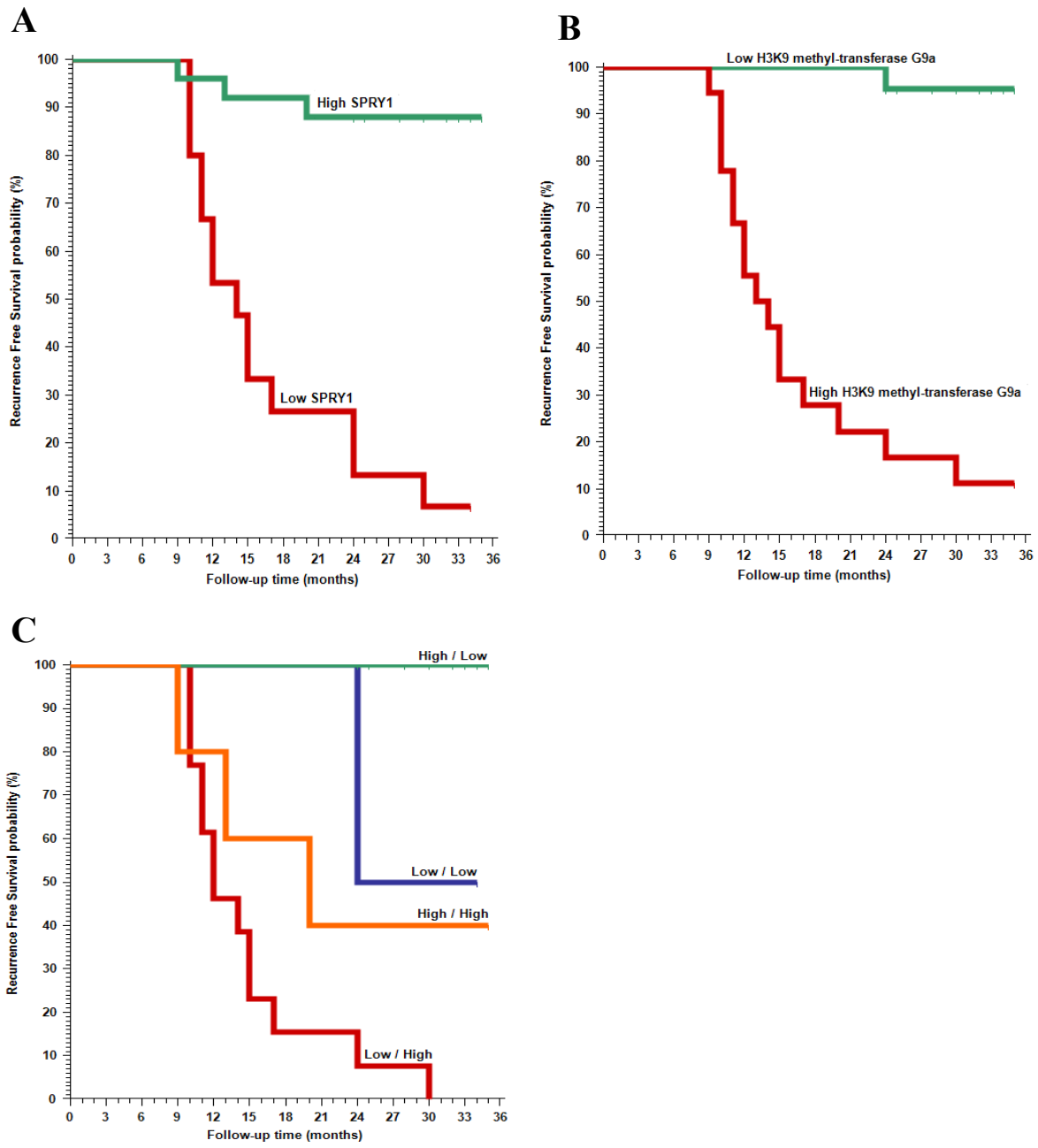

Figure 3: Kaplan-Meier plot of recurrence free survival: (A) stratified according to SPRY1, (B) stratified according to H3K9 methyl-transferase G9a, (C) stratified according to SPRY1 and H3K9 methyl-transferase G9a.

expression of Spourty 1 may increase EMT markers or EMT-inducing transcription factors and increasing the incidence of metastases and associated with poor prognosis.

These opposing results were explained by that different cell lines showed different epithelial and EMT markers changed following Spourty expression. Colon cancer cell lines differ in many genetic alterations. This is evidenced by their different growth rates and response to various drugs that leads to different response to changes in Spourty 1 expression as these cells also differ in Spourty substrates and Spourty regulators [33].

The pattern of alteration in Spourty 1 expression is not similar across the whole range of CRC studied and some studies found that diminished expression of some of Spourty in colon cancer reporting elevated expression of Spourty 1 in hepatocellular carcinoma (HCC), but it proved that Spourty 1 mRNA was found insignificant when the Spry1 expression in HCC tissues was compared with its expression in cirrhotic tissue, thereby implicating other causes, including aberrant hepatocyte function, in up regulation of Spry [34].

In conclusion, we found an inverse relationship between the expression of H3K9 methyl-transferase G9a and sprouty 1in CRC and that the combination of both markers give highly significant benefit in expecting its prognosis, as cases of colorectal cancer with unfavourable

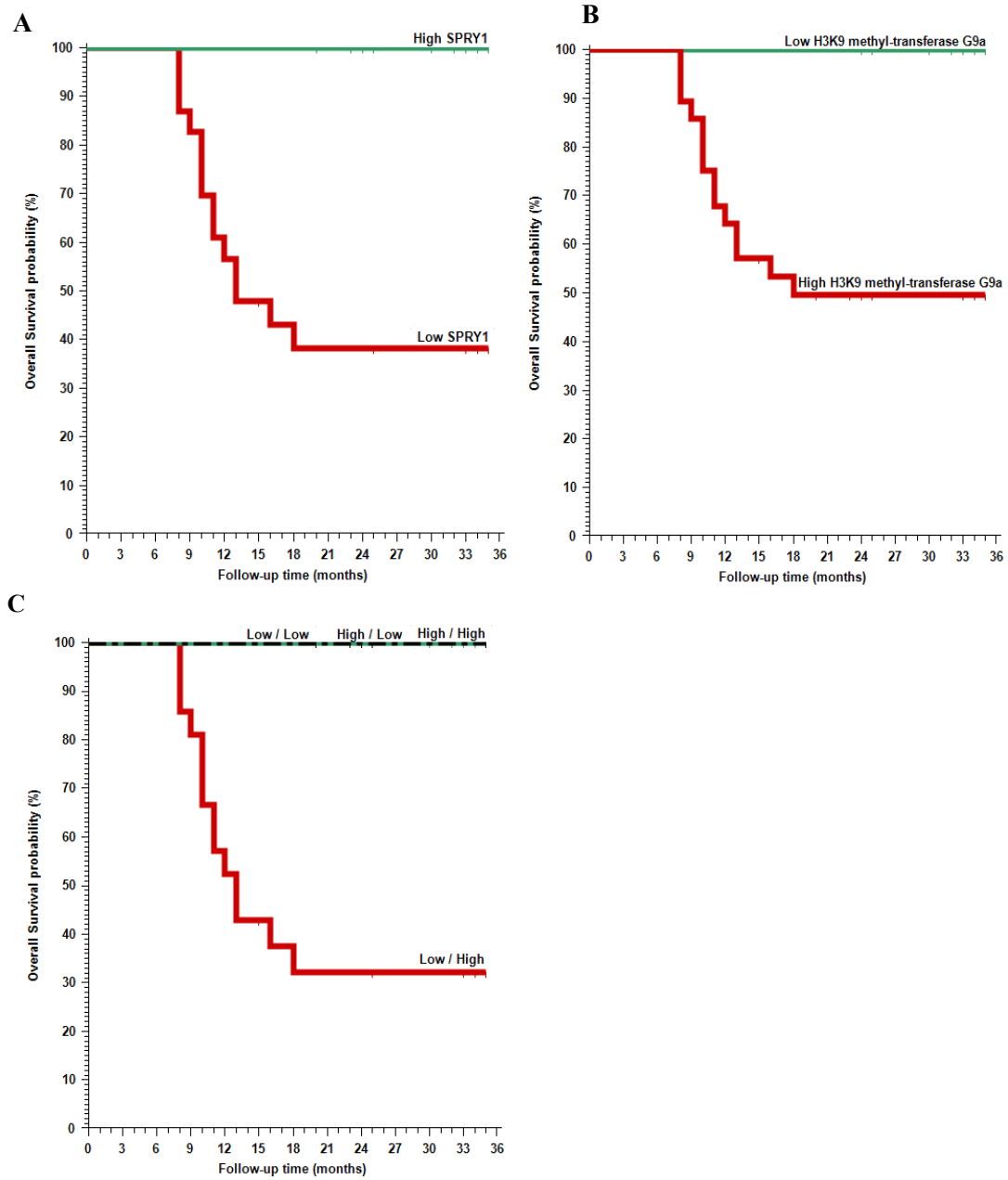

Figure 4: Kaplan-Meier plot of overall survival: (A) stratified according to SPRY1, (B) stratified according to H3K9 methyl-transferase G9a, (C) stratified according to SPRY1 and H3K9 methyl-transferase G9a.

prognosis had low expression of Spourty 1 and high expression of H3K9 methyl-transferase G9a that may help to find new therapeutic agents aiming to down regulating H3K9 methyl-transferase G9a and/or stimulation of sprouty 1 in such type of cancer to decrease spread and metastases of CRC and improving its prognosis.

Other studies regarding the expression of both markers in large number of cases of CRC and different types of cancers to prove and highlight our results.

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