ISSN- O: 2458 - 868X, ISSN–P: 2458 – 8687
Index Copernicus Value: 49. 23
PubMed - National Library of Medicine - ID: 101731606

International Journal of Medical Science and Innovative Research (IJMSIR)
IJMSIR : A Medical Publication Hub
Available Online at: www.ijmsir.com
Volume – 3, Issue –4, August - 2018, Page No. : 24 - 34

Expression and Clinical Significance of Sox9 in Colorectal Carcinoma

[1]Dr Madhvi, Junior Resident, Department of Pathology, KGMU, Lucknow
[2]Dr Riddhi Jaiswal, Associate Professor Department of Pathology, KGMU, Lucknow
[3]Dr Abhijeet Chandra, Professor, Department of Gastrosurgery, KGMU, Lucknow
[4]Dr Malti K Maurya, Associate Professor Department of Pathology, KGMU, Lucknow

Correspondence Author: Dr Riddhi Jaiswal, Associate Professor Department of Pathology, KGMU, Lucknow

Type of Publication: Original Research Paper

Conflicts of Interest: Nil

Abstract

Colorectal cancer is the third most common cancer in men and the second in women worldwide. CRC patients usually present with chronic abdominal pain, change in bowel habits, alternating episodes of diarrhoea and constipation, rectal bleeding or blood in stool, unexplained weight loss etc. Colorectal cancers result from the progressive accumulation of genetic and epigenetic alterations that lead to the transformation of normal colonic epithelium to colonic adenocarcinoma. At least two distinct pathways have been known; APC/β catenin pathway (Wnt pathway) also known as classical adenoma –carcinoma sequence and microsatellite instability pathway.

SOX9 directly regulates *IGFBP-4* in the intestinal epithelium. SOX9 regulates cell lineage specification by directly regulating target genes and previous reports have shown cell proliferative and suppressive roles for SOX9. SOX9 shares 70% amino acid homology to SRY through its HMG box, the domains of which are involved in the regulation of DNA-dependent processes, such as transcription and replication. It has important functional and clinical role in tumorigenesis.

The present study was carried out with the aim to demonstrate immunostaining for SOX9 in Colorectal carcinoma and its correlation with clinicopathological factors. A total of 60 cases of colorectal carcinoma were taken into study. Histological sections were reviewed and graded according to differentiation.

Keywords: SOX9, Colorectal cancer, Diagnosis

Introduction

Data consolidated from reports of 27 population-based and 17 hospital-based registries for three consecutive years from cancer registries in India was presented at a workshop at the Indian Council of Medical Research which showed emerging trends in cancer incidence in India, such as the increase in lung cancer in women and colon cancer in men. **[1]** Surveillance, epidemiology and end result program estimated 1,32,700 new cases of Colorectal cancer (CRC) worldwide and resulting in 49,700 deaths due to cancer (approximately 8.4%) **[2].** It is the second most common cause of cancer in women (9.4% of all cancer diagnoses) and the third most common in men (10.0% of all cancers) **[3].** The incidence of CRC is still several folds lower in India than in most developing and developed countries and there is no scientific evidence for starting a CRC screening program in India **[4].**

Colorectal carcinomas are derived from the intestinal epithelium, which is maintained by epithelial homeostasis. It typically starts as a benign tumor which over time

becomes adenomatous, dysplastic and then cancerous. Particularly, genes constituting the Wnt signaling pathway are crucial for the maintenance of undifferentiated progenitors in the crypts and for the maintenance of the post mitotic Paneth cells **[5].** It is becoming increasingly clear that mutations in developmentally regulated genes can cause the initiation and progression of these cancers. Wnt activating mutations are mostly found in colorectal cancers, frequently targeting the tumor suppressors APC or Axin2 or the oncogene β-catenin. Members of other important developmental programs such as Notch and BMP are also deregulated in colorectal cancers **[6].** Adenocarcinoma usually account for 98% of cases of colorectal carcinoma, rarely it can be squamous cell carcinoma, adenosquamous, neuroendocrine and lymphomas. In the early 90s, the hereditary cancer syndrome Familial Adenomatous Polyposis (FAP) was discovered to be directly linked to mutations within the adenomatous polyposis coli gene (APC). Two years later, Morin et al, 1997 **[7]** found a close interaction between APC and β-catenin was demonstrated, and as APC mutations were found at high frequencies in colorectal adenomas and carcinomas, it was soon realized that the *Wnt/β-catenin signaling* pathway plays an initiating and rate-limiting role in colorectal tumorigenesis. More recently, large-scale exome-sequencing efforts have confirmed that Wnt/β-catenin signaling is deregulated in colorectal carcinomas.

In 1990, Fearon and Vogelstein et al proposed a multistep genetic model of colorectal carcinogenesis in which inactivation of the adenomatous polyposis coli (APC) tumor suppressor gene located on chromosome 21 occurs first in normal colonic mucosa referred to as “first hit” followed by activating mutations in the KRAS gene and subsequent additional mutations (e.g., DCC, TP53 , and TGF - β pathway genes) **[8].** Other mutations include K-RAS, DCC deletion and inactivation of TP53 occuring in a sequence lead to development of cancer.

ICMR concluded that tumors of the colon and rectum can be grouped into one and they identified a set of 24 genes mutated in a significant number of cases with emphasis on new genes such as SOX9 and IGF2 [**4, 5].** These genes were involved in regulating cell proliferation in bowel and can therefore serve as potential therapeutic drug targets.

Notch signaling pathways are also deregulated in colorectal cancers, especially in chemotherapy resistance. Notch signaling induces epithelial to mesenchymal transition (EMT), proliferation, metastasis and activates Wnt pathway. SOX9 is also believed to be notch induced transcription factor which accumulates in the nucleus and causes transcriptional regulation of downstream targets. **[9]**

Role of Sox9 in Colorectal Carcinoma

Sex determining region Y (SRY)-related high mobility group (HMG)-box 9 (SOX9), as a developmental transcription factor, plays a vital role in the regulation of sex determination, cartilage development, intestinal differentiation and adult progenitor cell pool maintenance. SOX9 shares 70% amino acid homology to SRY through its HMG box, the domains of which are involved in the regulation of DNA-dependent processes, such as transcription and replication [**10].**

Sox9 plays a critical role in the homeostasis of colorectal epithelium. In particular, it is localized in proliferating bottom third of Lieberkuhn crypt cells and promotes stem/progenitor cell proliferation and it is also required for Paneth cell differentiation in the intestinal epithelium during development and in adult stages. SOX9 is a transcription factor and a downstream target of Wnt/β-catenin signaling with possible roles in β-catenin regulation **[5,11,12**]. Deregulation of SOX9 has been reported for several cancers and recent large-scale exome-

sequencing efforts have revealed that SOX9 is mutated in a subset of colorectal carcinomas.

Blache and colleagues [13] first reported that SOX9 can also inhibit intestinal crypt differentiation in the colon by repressing two intestine-specific differentiation genes, *CDX2* and *MUC2*. Recent studies indicated that SOX9 is required for Paneth cell differentiation in the intestinal epithelium. It is also overexpressed in various other human cancers such as melanoma, breast cancer, glioma, osteosarcoma, stomach and prostate cancer. The prognostic potential of SOX9 has only been evaluated in one adequate dataset, which suggested that high expression of the SOX9 protein was associated with an adverse prognosis.

Microsatellite instability (MSI) is the condition of genetic hypermutability resulting from impaired DNA mismatch repair (MMR). MMR corrects errors that occur during DNA replication such as deletions and short insertions. The proteins involved in MMR correct the errors by binding to mismatched sections of DNA and adding the correct sequence. MSI results from defect in MMR proteins. This can be due to mutations or oxidative stress. Role of transcription factors like β-catenin, SOX9 have been studied by Panza A et al (2013) and levels of SOX9 were upregulated at both mRNA and protein level in MSI **[14]**

The TCF/LEF transcription factors share a common high mobility group DNA binding domain with the SOX transcription factors. Similarly to TCFs, SOX proteins have been widely implicated in the establishment of cell multipotency and cell commitment (Mori-Akiyama et al., 2003) **[15].**

Material and Methods

The study was conducted in the Department of Pathology at King George's Medical University, Lucknow, U.P. Both retrospective and prospective specimens were included.

All cases of colorectal carcinoma received in the past three years were included in the study. Few cases of normal bowel were also collected to see immunohistochemical staining pattern and to study the expression. The old cases were taken out from the records of department of pathology and along with the new case, were followed up for survival. The clinical data of the cases were recorded from the registration forms. Hematoxylin and Eosin (H&E) stained slides and blocks were retrieved from the departmental records. Gross specimens were also retrieved as and when required. All the slides available were reviewed to study the histological diagnosis, TNM staging, lympho vascular and peri neural invasion. 3-4 micrometer thick sections were taken from each block for *H & E staining* and *SOX9 immuno-histochemical (IHC) staining* on 3 amino propyl tri-ethoxysilane coated slides using specific rabbit monoclonal antibody to SOX9 [EPR14335] (CAT NO. ab185230) provided by Abcam manufacturer . IHC was done using Novolink Mini polymer detection system (Leica) as per following protocol.

Adenocarcinoma were staged and histologically graded according to the criteria of American Joint committee on cancer (AJCC), 7th edition; 2013,protocol for reporting carcinoma of the colon and rectum. Patients were followed up ranging from 6 months to 2 years. All Patients or their relatives were contacted with the help of department records and their status, live or dead was confirmed.

Presence of brown coloured end product at the site of target antigen was indicative of positive reactivity for SOX9. The percentage of positive tumor cells for SOX9 were scored as:

- 1+ : 0-25%
- 2+ : 26-50%
- 3+ : >50%

The staining intensity was recorded as "Low intensity" and "High intensity". The nuclear and cytoplasmic positivity was also recorded per individual case.

The result analysis was carried out by using SPSS 16.0 version (Chicago, Inc., USA)

Observations and Results

Table-1: Distribution of result of histopathology

Histopathology	No. (n=60)	%
Well Differentiated	23	38.3
Moderately Differentiated	21	35.0
Poorly Differentiated	4	6.7
Mucinous	12	20.0

Table-2: Association of histopathology with age and sex of the patients.

	No. of patients	Histopathology								p-value [1]
		WD		MD		PD		Mucinous		
		No.	%	No.	%	No.	%	No.	%	
Age in years										
<40	15	4	26.7	5	33.3	1	6.7	5	33.3	0.47
≥40	45	19	42.2	16	35.6	3	6.7	7	15.6	
Sex										
Male	38	15	39.5	13	34.2	3	7.9	7	18.4	0.93
Female	22	8	36.4	8	36.4	1	4.5	5	22.7	

[1]Chi-square test

Table 2: The percentage of WD (42.2%) and MD (35.6%) were higher among the age ≥40 years compared to <40 years. The percentage of PD was similar in both the age groups. WD was higher among males (39.5%) than females (36.4%). There was no significant (p>0.05) association of histology with age and sex.

Table-3: Association of histopathology with site.

Site	No. of patients	Histopathology								p-value[1]
		WD		MD		PD		Mucinous		
		No.	%	No.	%	No.	%	No.	%	
Caecum	5	2	40.0	0	0.0	0	0.0	3	60.0	0.06
Ascending colon	21	9	42.9	8	38.1	3	14.3	1	4.8	
Transverse colon	6	1	16.7	5	83.3	0	0.0	0	0.0	
Descending colon	8	4	50.0	2	25.0	0	0.0	2	25.0	
Rectum	11	5	45.5	2	18.2	1	9.1	3	27.3	
Colorectal	4	2	50.0	0	0.0	0	0.0	2	50.0	
Anorectal	5	0	0.0	4	80.0	0	0.0	1	20.0	

[1]Chi-square test

Table-3 & Fig.3 shows the association of histopathology with site. Rectum (50%) was observed to be more common in WD whereas, transverse colon (83.3%) was most prevalent in MD. Caecum (60%) was found to be more common in mucinous.

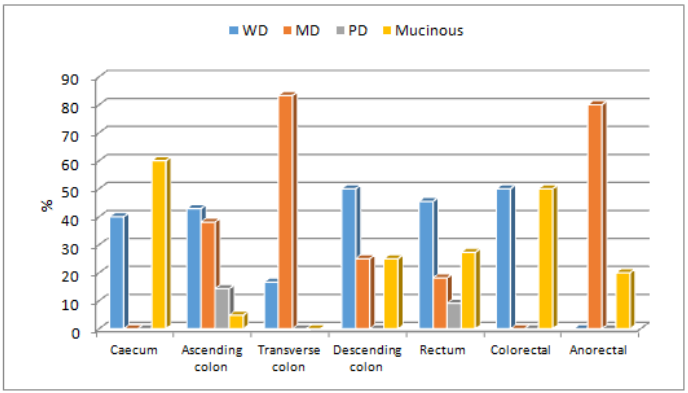

Fig.1: Association of histopathology with site

Table-4: Association of SOX9 with age and gender.

	No. of patients	Low expression		Strong expression		RR (95%CI), p-value[1]
		No.	%	No.	%	
Age in years						
<40	15	5	33.3	10	66.7	0.38
≥40	45	10	22.2	35	77.8	
Sex						
Male	38	9	23.7	29	76.3	0.86 (0.35-2.11), 0.75
Female	22	6	27.3	16	72.7	1.00 (Ref.)

RR-Relative risk, CI-Confidence interval, Ref: Reference, [1]Chi-square test

Table-4 shows the association of SOX9 with age and sex. The low expression was insignificantly (p>0.05) higher among the patients of age <40 (33.3%) than ≥40 (22.2%). The low expression was 14% insignificantly lower among males than females (RR=0.86, 95%CI=0.35-2.11, p=0.75).

Table-5: Association of SOX9 with Clinicopathological features

	No. of patients	Low expression		Strong expression		RR (95%CI), p-value[1]
		No.	%	No.	%	
Blood in stool						
Present	40	12	30.0	28	70.0	2.00 (0.63-6.28), 0.20
Absent	20	3	15.0	17	85.0	1.00 (Ref.)
Habits						
Constipation	51	14	27.5	37	72.5	2.47 (0.36-16.53), 0.29
Diarrhoea	9	1	11.1	8	88.9	1.00 (Ref.)
Anorexia						
Present	50	12	24.0	38	76.0	0.80 (0.27-2.32), 0.68
Absent	10	3	30.0	7	70.0	1.00 (Ref.)
Risk Factors						
Tobacco user	20	4	20.0	16	80.0	0.83
Alcohol user	12	3	25.0	9	75.0	
DM	5	2	40.0	3	60.0	
None	23	6	26.1	17	73.9	
Dietary habit						
Vegetarian	22	6	27.3	16	72.7	1.15 (0.47-2.80), 0.75
Non-vegetarian	38	9	23.7	29	76.3	1.00 (Ref.)
Polyp						
Prior ulcerative colitis	4	0	0.0	4	100.0	0.51
Prior crohn's disease	2	0	0.0	2	100.0	
Any polyp	3	1	33.3	2	66.7	
None	51	14	27.5	37	72.5	
Family history						
Present	6	3	50.0	3	50.0	2.25 (0.87-5.77), 0.13
Absent	54	12	22.2	42	77.8	1.00 (Ref.)

RR-Relative risk, CI-Confidence interval, Ref: Reference,

[1]Chi-square test

Table-5 None of the features were found to be associated with SOX9 (p>0.05).

Table-6: Association of histopathology with SOX9

SOX9	No. of patients	Histopathology								p-value[1]
		WD		MD		PD		Mucinous		
		No.	%	No.	%	No.	%	No.	%	
Low expression	15	0	0.0	3	20.0	3	20.0	9	60.0	0.001*
Strong expression	45	23	51.1	18	40.0	1	2.2	3	6.7	

[1]Chi-square test, *Significant

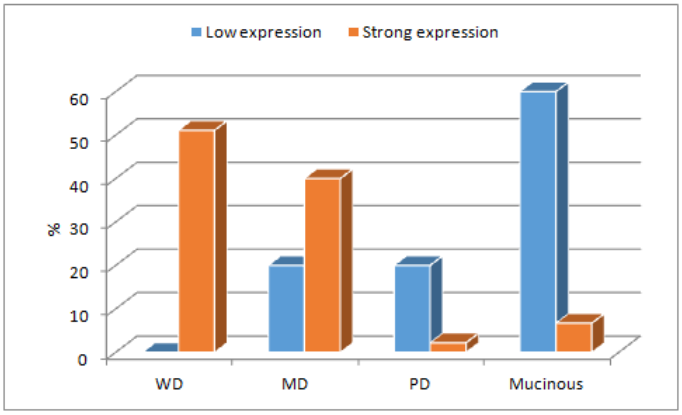

Fig 2: Association of histopathological grading with SOX9

Table-6 & Fig.2 show the association of histopathology with SOX9. There was significant (p=0.001) association of histological grading with SOX9.

Table-7: Association of histopathology with SOX9 location.

EXP	No. of patients	Histopathology								p-value[1]
		WD		MD		PD		Mucinous		
		No.	%	No.	%	No.	%	No.	%	
Nuclear	56	23	41.1	21	37.5	0	0.0	12	21.4	0.001*
Cytoplasmic	4	0	0.0	0	0.0	4	100.0	0	0.0	

[1]Chi-square test, *Significant

Table-7 & Fig.3 shows the association of histopathology with location. Nuclear was found in 41.1% of WD, 37.5 of MD and 21.4% of mucinous. The difference was found to be statistically significant (p=0.001).

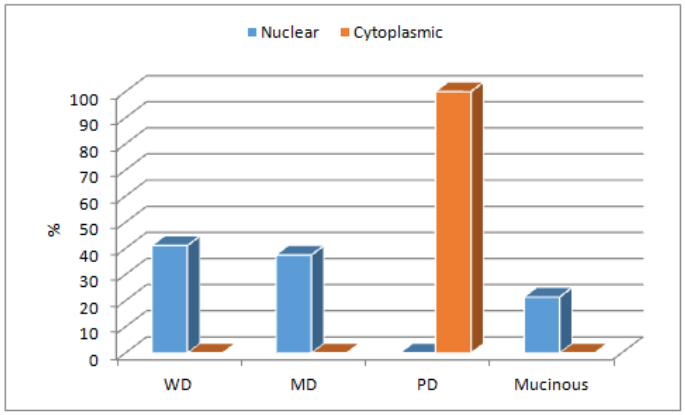

Fig.3: Association of histopathology with location of expression

Table-8: Association of SOX9 with TNM staging.

	No. of patients	Low expression		Strong expression		p-value[1]
		No.	%	No.	%	
T						
T 2	19	7	36.6	12	63.2	0.01*
T 3	31	6	19.4	25	80.6	
T 4	10	2	20.0	8	80.0	
Nodes						
N0	33	8	24.2	25	75.8	0.52
N1a	10	2	20.0	8	80.0	
N1b	5	2	40.0	3	60.0	
N2a	1	0	0.0	1	100.0	
N2b	1	1	100.0	0	0.0	
Nx	10	2	20.0	8	80.0	
Metastatis						
M0	50	12	24.0	38	76.0	0.50
M1a	8	3	37.5	5	62.5	
M1b	2	0	0.0	2	100.0	

[1]Chi-square test, *Significant

Table-8 shows the association of SOX9 with TNM staging. T was found to be significantly associated with SOX9 (p=0.01).

Table-9: Association of SOX9 with CAP staging

	No. of patients	Low expression		Strong expression		p-value[1]
		No.	%	No.	%	
I	1	0	0.0	1	100.0	0.87
II	39	9	23.1	30	76.9	
III	10	3	30.0	7	70.0	
IV	10	3	30.0	7	70.0	

[1]Chi-square test

Table-9 & Fig. 4 shows the association of SOX9 with staging. Staging was found to be insignificantly associated with SOX9 (p>0.05).

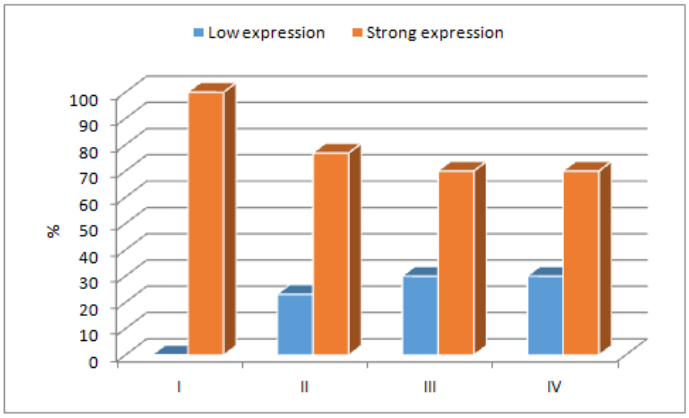

Fig.4: Association of SOX9 with CAP staging

Table-10: Association of SOX9 with survival

Survival	No. of patients	Low expression		Strong expression		RR (95%CI), p-value[1]
		No.	%	No.	%	
Alive	30	4	13.3	26	86.7	0.36 (0.13-1.90), 0.07
Dead	30	11	36.7	19	63.3	1.00 (Ref.)

RR-Relative risk, CI-Confidence interval, Ref: Reference, [1]Chi-square test

Table-10 shows the association of SOX9 with mortality. The survival was 64% lower in low expression, however, association was found to be statistically insignificant (RR=0.36, 95%CI=0.13-1.90,p=0.07).

Discussion

Colorectal cancer is one of the most aggressive cancers worldwide. Curing this disease and to decrease its burden will require the identification of molecular biomarkers for prognosis or novel targets for therapeutic intervention. SOX9 is a new emerging immunomarker and is being analysed in various carcinomas of human body by various investigators.

Charbel Darido et al (2007) analysed the hypothesis that defective Claudin-7 regulation by Tcf-4 and Sox-9 disrupts the polarity and increases the tumorigenicity of colorectal cancer cells. Claudin-7 was strongly expressed in the intestine of Sox-9–deficient mice and in CRC cells with low Sox transcriptional activity. Sox-9 overexpression in these cells reinstated claudin-7 repression, and residual claudin-7 was no longer localized along the basolateral membrane, but was instead restricted to tight junctions. They found that Sox-9–induced polarization was completely reversed after virus-mediated claudin-7 overexpression. Their results indicate that Tcf-4 maintains low levels of claudin-7 at the bottom of colonic crypts, acting via Sox-9. This negative regulation seems to be defective in CRC, possibly due to decreased Sox-9 activity, and the resulting claudin-7 overexpression promotes a loss of tumor cell polarization and contributes to tumorigenesis **[16]** .

Chun-Hui Zhou et al (2012) analysed clinical significance of SOX9 in human non-small cell lung cancer (NSCLC), its progression and overall patient survival **[17].**

Chakravarty et al. (2011) demonstrated that cytoplasmic SOX9 may serve as a valuable prognostic marker for invasive ductal carcinomas and metastatic breast cancer, and its significant correlation with breast tumor cell proliferation implied that SOX9 may directly contribute to the poor clinical outcomes associated with invasive breast cancer **[18].**

Haibo Zhu et al indicated that Sox9 is upregulated in osteosarcoma and is associated with poor outcome in patients **[19].**

Bingjian et al (2008) analysed the role of SOX9 in colorectal carcinomas. They correlated the expression of SOX9 and β-catenin by immunohistochemical staining , quantitative real-time reverse transcription–polymerase chain reaction (Q-PCR) and Western blot in colorectal cancer patients (n= 188), of which 61 patients had colonic and 127 had rectal cancer. They also analysed the clinicopathological parameters with the immunohistochemical expression. All patients underwent tumor resection with tumor-free margins. 96 patients were given adjuvant 5-fluorouracil-based chemotherapy and 92 did not receive any chemotherapy. Surviving patients were observed. Of the study patients, 59 died of cancer and 55 patients survived. Immunostaining results showed more SOX9+ cells in the lower zone of colonic crypts than in the upper zone in the normal bowel sections ($P < .05$). Their study consistently confirmed that SOX9 is up-regulated in CRC compared with normal mucosa ($P < .05$). Strong SOX9 expression and nuclear β-catenin were less commonly seen in mucinous adenocarcinoms and signet-ring cell carcinoma (mucin-producing carcinoma) than in non–mucin-producing colorectal cancer ($P < .05$; $\chi 2$ test). They found that strong SOX9 expression was not significantly associated with high-grade histologic features ($P > .05$). Strong SOX9 expression showed a lower overall 5-year survival in colorectal cancer (strong expression, 40% [17/43] vs low expression, 69% [66/95]; $P < .01$). The Cox proportional hazards model showed that strong SOX9 expression was an independent adverse

prognostic indicator in colorectal cancer (P < .05). They came to conclusion that detection of SOX9 expression might contribute to predicting clinical outcome of patients **[20].**

P A Candy et al (2010) evaluated the expression of Notch-induced transcription factors (NTFs) HEY1, HES1 and SOX9 in colorectal cancer (CRC) patients to determine their clinicopathologic and prognostic significance. Levels of HEY1, HES1 and SOX9 protein were measured by immunohistochemistry in a nonmalignant and malignant tissue microarray of 441 CRC patients, and the findings were correlated with pathologic, molecular and clinical variables. They concluded that NTFs HEY1, HES1 and SOX9 were overexpressed in tumours relative to colonic mucosa. They concluded that tumour overexpression of SOX9 correlated with markedly poorer survival but had no predictive effect in untreated patients **[21].**

Ander Mathew et al tested SOX9, a high mobility group (HMG) box transcription factor, plays critical roles during embryogenesis and its activity is required for development, differentiation and lineage commitment in various tissues including the intestinal epithelium. In this study, gain of SOX9 copy number in some primary colorectal cancers was found. SOX9 exhibited several pro-oncogenic properties, including the ability to promote proliferation, inhibit senescence and collaborate with other oncogenes in neoplastic transformation. In colorectal cancer cells, SOX9 expression facilitated tumor growth and progression whilst its inactivation reduced tumorigenicity. They found a positive correlation between expression levels of SOX9 and BMI1 and a negative correlation between SOX9 and ARF in clinical samples. Taken together, their findings provide direct mechanistic evidence of the involvement of SOX9 in neoplastic pathobiology, particularly in colorectal cancer[**22, 23].**

Anna Panza et al studied the interplay between SOX9, β-catenin and Peroxisome Proliferator-Activated Receptor γ **(**PPARγ**)** activation in colorectal cancer. They evaluated SOX9, β-catenin and PPARγ expression concluded that tumour overexpression of SOX9 correlated with markedly poorer survival but had no predictive effect in untreated patients **[14].**

Different gene expression profiles in colorectal cancer (CRC) and their correlation between candidate genes and clinical histopathological features, as well as in developing prognostic prediction markers have also been syudied. Results showed VEGF (71.4%), **SOX9 (68.8%)**, TP53 (59.4%) as significantly overexpressed genes in CRC tissues and were associated with poor histological features **[24].**

Together, these observations suggest that SOX9 may be an attractive candidate marker that is involved in the tumor initiation and tumor progression, and it may play different roles depending on cancer types.

In our study 8/23of Well Differentiated (WD) patients, 13/21 of Moderately Differentiated (MD), 2/2 of Poorly Differentiated (PD) and 7/12 mucinous carcinoma patients died within follow up period of maximum 2 years. We correlated the expression of SOX9 with survival of patients, where we did not find any significant correlation (p=0.07). However we found that most of the patients who survived (86.7%) presented strong immunoexpression (p=0.07) and were mostly of well differentiated histology grade.

The results of our study imply a promising impact of SOX9 immunostaining in the various differentiation of colorectal carcinoma with statistically significant values.

The present study was limited by factors like comparatively small sample size, less availability of data in records and poorly differentiated tumors for comparison. This requires immunohistochemical and molecular evaluation of this marker on a large population of CRC and a long term follow up.

Conclusions

- Colorectal carcinoma is more common in 4th to 5th decade of life. The mean age of presentation of colorectal carcinoma is 49.02 years.
- Patients less than 40 years of age presented with higher histological grades (33.3%) and mucinous adenocarcinoma (33.3%) compared to low grade (well differentiated) tumors (26.7%).
- SOX9 expression showed statistically highly significant correlation with histological grading of colorectal carcinoma (p=0.001). Strong nuclear positivity was confirmed in well differentiated (51.1%), moderately differentiated (40%), poorly differentiated (2.2%) and mucinous adenocarcinoma (6.7%).
- All the cases of adenocarcinoma (WD) showed strong nuclear positivity (23/23). Cytoplasmic SOX9 expression was observed in all cases of poorly differentiated tumors (4/4). All poorly differentiated carcinoma cases showed appearance of cytoplasmic expression (p=0.001). These findings suggest the nuclear sequestration of SOX9 into cytoplasm with high grading of tumor. These findings suggest its role in spread and invasiveness of tumor. However a larger population based study and molecular tests evaluations are needed to study the above hypothesis.
- There was no significant correlation between expression of SOX9 with clinicopathological factors and TNM/CAP staging.
- SOX9 expression was not significantly related to survival of patients.
- In particular SOX9, have a very real potential to enter routine clinical practice in defining the utility of adjuvant chemotherapy for individual patients.

References

1. Day LW, Cello JP, Somsouk M, Inadomi JM. Prevalence of gastric cancer versus colorectal cancer in Asians with a positive fecal occult blood test. Indian J Gastroenterol. 2011;30:209–16.
2. World Cancer Report 2014. World Health Organization. 2014. pp. Chapter 1.1.ISBN 9283204298.
3. Mallath MK, Taylor DG, Badwe RA, Rath GK, Shanta V, Pramesh CS, et al. The growing burden of cancer in India: epidemiology and social context. Lancet Oncol. 2014;15:e205–e12
4. Kucherlapati, et al. “Comprehensive molecular characterisation of colon and rectal cancer.” Nature 487(2012): 330-337
5. Ali R, Barnes I, Kan SW, Beral V. Cancer incidence in British Indians and British whites in Leicester, 2001–2006. Br J Cancer. 2010;103:143–
6. Bastide P, Darido C, Pannequin J, Kist R, Robine S, Marty-Double C et al. Sox9 regulates cell proliferation and is required for Paneth cell differentiation in the intestinal epithelium. The Journal of Cell Biology. 2007;178(4):635-648.
7. Morin, P.J., A.B. Sparks, V. Korinek, N. Barker, H. Clevers, B. Vogelstein, and K.W. Kinzler. 1997. Activation of β -catenin-Tcf signaling in colon cancer by mutations in β-catenin or APC. Science. 275:1787–1790.
8. Fearon E, Vogelstein B. A genetic model for colorectal tumorigenesis. Cell. 1990;61(5):759-767
9. Shi Z, Chiang C, Labhart P, Zhao Y, Yang J, Mistretta T et al. Context-specific role of SOX9 in NF-Y mediated gene regulation in colorectal cancer cells. Nucleic Acids Res. 2015;43(13):6257-6269
10. Barrionuevo F, Scherer G: SOX E genes: SOX9 and SOX8 in mammalian testis development. Int J Biochem Cell Biol 2010, 42:433–436.

11. Muto T, Bussey HJ, Morson BC. The evolution of cancer of the colon and rectum. Cancer1975;36(6):2251–2270
12. S. Dupasquier, R. Abdel-Samad, R.I. Glazer, P. Bastide, P. Jay, D. Joubert, V. Cavaillès, P. Blache, C. Quittau-Prévostel, A new mechanism of SOX9 action to regulate PKCalpha expression in the intestine epithelium, J. Cell Sci. 122 (2009) 2191–2196.
13. Blache P, van de Wetering M, Duluc I, Domon C, Berta P, Freund J et al. SOX9 is an intestine crypt transcription factor, is regulated by the Wnt pathway, and represses the CDX2 and MUC2 genes. The Journal of Cell Biology. 2004;166(1):37-47
14. Panza A, Pazienza V, Ripoli M, Benegiamo G, Gentile A, Valvano M et al. Interplay between SOX9, Î²-catenin and PPARÎ³ activation in colorectal cancer. Biochimica et Biophysica Acta (BBA) - Molecular Cell Research. 2013;1833(8):1853-1865
15. Mori Akiyama Y, van den Born M, van Es J, Hamilton S, Adams H, Zhang J et al. SOX9 Is Required for the Differentiation of Paneth Cells in the Intestinal Epithelium. Gastroenterology. 2007;133(2):539-546.
16. Darido C, Buchert M, Pannequin J, Bastide P, Zalzali H, Mantamadiotis T et al. Defective Claudin-7 Regulation by Tcf-4 and Sox-9 Disrupts the Polarity and Increases the Tumorigenicity of Colorectal Cancer Cells. Cancer Research. 2008;68(11):4258-4268.
17. Zhou C, Ye L, Ye S, Li Y, Zhang X, Xu X et al. Clinical significance of SOX9 in human non-small cell lung cancer progression and overall patient survival. J Exp Clin Cancer Res. 2012;31(1):18
18. Chakravarty G, Moroz K, Makridakis N, Lloyd S, Galvez S, Canavello P et al. Prognostic significance of cytoplasmic SOX9 in invasive ductal carcinoma and metastatic breast cancer. Experimental Biology and Medicine. 2011;236(2):145-155
19. Zhu H, Tang J, Tang M, Cai H. Upregulation of SOX9 in osteosarcoma and its association with tumor progression and patientsâ€™ prognosis. Diagnostic Pathology. 2013;8(1):183
20. Bingjian Fang Y, Xu J, Wang L, Xu F, Xu E et al. Analysis of SOX9 Expression in Colorectal Cancer. American Journal of Clinical Pathology. 2008;130(6):897-904
21. Candy P, Phillips M, Redfern A, Colley S, Davidson J, Stuart L et al. Notch-induced transcription factors are predictive of survival and 5-fluorouracil response in colorectal cancer patients. Br J Cancer. 2013;109(4):1023-1030.
22. Mathew A, Collado M, Wise C, Manterola L, Cekaite L, Tye A et al. Oncogenicity of the Developmental Transcription Factor Sox9. Cancer Research. 2012;72(5):1301-1315.
23. Jussawalla DJ, Gangadharan P. Cancer of the colon: 32 years of experience in Bombay, India. J Surg Oncol. 1977;9:607–22
24. Liu J, Shang Guan Y, Qi Y, Wang H, Zhang T, Zhou C. The evaluation of SOX9 expression and its relationship with carcinoembryonic antigen-related cell adhesion molecule 1 in gastric neoplastic and nonneoplastic lesions. Annals of Diagnostic Pathology. 2012;16(4):235-244

Microphotographs

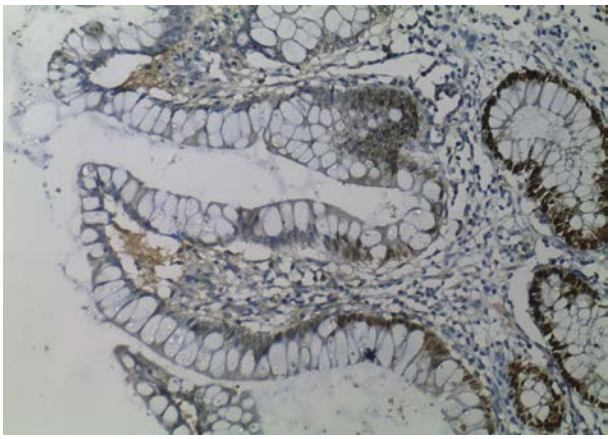

Fig. 1: Nuclear expression of SOX9 in lower one third of crypt in normal bowel mucosa x 400X

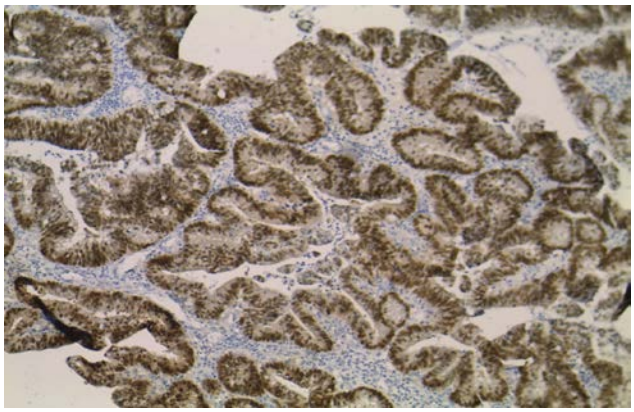

Fig. 2: Strong nuclear expression of SOX9 in adenocarcinoma (WD) x 100X

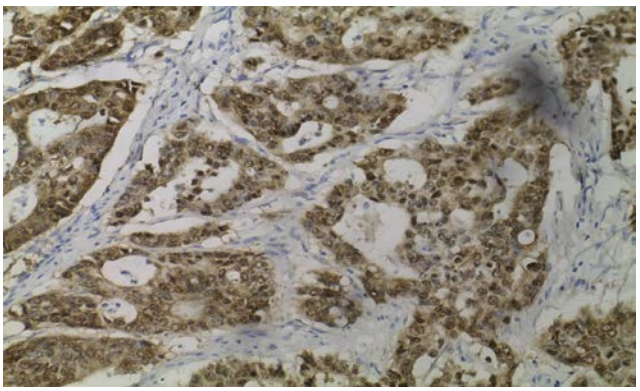

Fig. 3: Low nuclear expression of SOX9 in adenocarcinoma (MD) x 200X

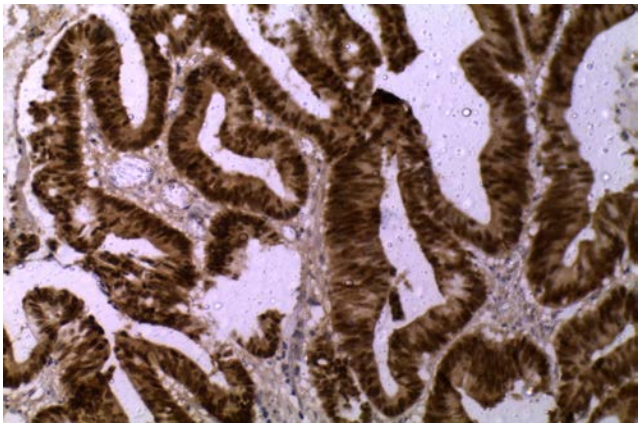

Fig. 4: Strong nuclear expressions of SOX9 in adenocarcinoma (MD) x 200X

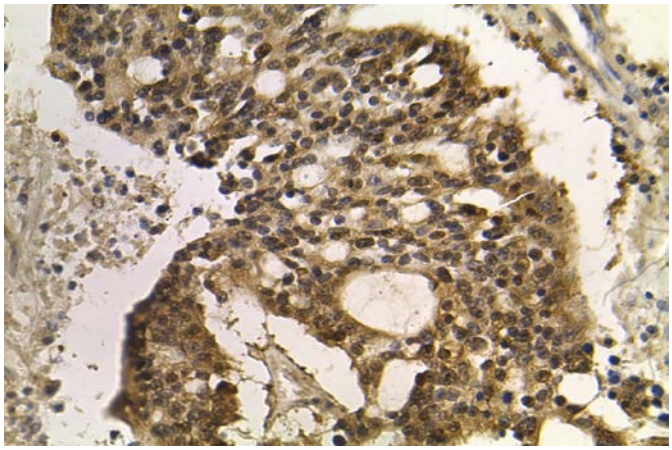

Fig. 5: Strong nuclear and cytoplasmic expression of SOX9 in adenocarcinoma (PD) x 200X

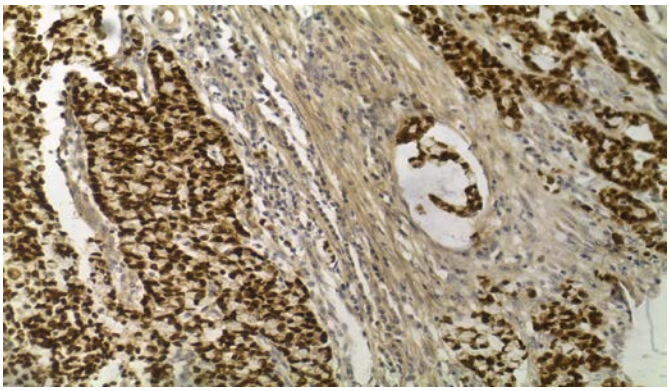

Fig. 6 Strong nuclear expression of SOX9 in mucinous adenocarcinoma x 200X