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SURVIVIN EXPRESSION IN COLORECTAL ADENOCARCINOMAS

Pathology

Dr.S.Brihadisvarar Postgraduate, Department of Pathology, Govt. Stanley Medical College, Chennai.

Dr.Nalli.R.Sumitra Devi Professor of Pathology, Department of Pathology, Govt. Stanley Medical College, Chennai.

ABSTRACT

Introduction : Colorectal adenocarcinomas contribute to a significant proportion of cancer related morbidity and mortality. Survivin is a novel member of the IAP family of proteins involved in apoptosis inhibition, being overexpressed in many cancers. This study was done to evaluate the expression of Survivin in colorectal adenomas and adenocarcinomas and its association with the clinicopathological tumor characteristics.

Materials and methods : Immunohistochemical analysis of Survivin expression was performed on 30 cases each of normal colorectal mucosa, adenomas and adenocarcinomas respectively.

Results: Survivin expression was absent in normal mucosa with increased expression in adenomas and maximal expression in adenocarcinomas. It also correlated with the degree of differentiation of adenocarcinomas.

Conclusion : The results indicate that dysregulation and over expression of Survivin is involved in colorectal tumorigenesis and malignant transformation of adenomas.

KEYWORDS

Survivin, Colorectum, Adenoma, Adenocarcinoma,Immunohistochemistry.

Introduction:

With 1.2 million new cases being diagnosed every year, colorectal cancer accounts for over nine percent of newly diagnosed cancers, with around 1.2 million new cases and 600,000 associated deaths world wide, responsible for almost 10% of all cancer deaths.[1] It is mainly a disease of developed countries with a western culture with a comparatively lower incidence in Asian and African countries including India however recent studies have shown an increase in incidence in these regions.[2] Colorectal adenocarcinoma is the prototype malignancy where the sequence of transition from normal mucosa to carcinoma has been well documented, involving multiple genetic alterations at each level. One important mechanism in malignant transformation is dysregulation of apoptosis.

Survivin, a newly identified member of the Inhibitor of Apoptosis (IAP) family of proteins is a bifunctional regulator inhibiting apoptosis and involved in cellular proliferation.[3] It is normally expressed during embryonic development, nearly undetectable in terminally differentiated tissues and is over expressed in a multitude of cancers including colorectal carcinomas.[4]

In the present study the expression of Survivin by normal colonic mucosa, colorectal adenomas and adenocarcinomas was evaluated using immunohistochemistry and its relation to various clinico-pathological characters including tumor grade, stage, patient age and gender were analysed.

Materials and methods :

The study included 30 cases each of normal colonic mucosa, colorectal adenomas and adenocarcinomas. The age of the patients ranged from 10 years to 78 years. The patients had received neither chemotherapy nor radiotherapy prior to surgery. Those with recurrent disease and malignancies other than adenocarcinomas were excluded. Normal tissues were obtained from uninvolved resected margins of the specimens. The adenocarcinoma cases included 10 cases of well differentiated, 12 cases of moderately differentiated and 8 cases of poorly differentiated adenocarcinomas. 13 cases belonged to stage I, 8 cases to stage II and 9 cases to stage III respectively.

The specimens were fixed in 10% formalin, underwent routine tissue processing in automated histokinette and paraffin embedded. 5 Micron thick sections were cut using semiautomated microtome and stained using haematoxylin and eosin. The diagnosis was confirmed and suitable blocks were chosen for immunohistochemistry.

Immunohistochemical staining:

Sections for immunohistochemistry were also cut in semiautomatic microtome at 5 Micron thickness. Slides coated with chrome alum were used. Immunohistochemistry was performed by using HRP polymer technique. The slides were incubated overnight at 45 degree Celsius followed by incubation at 70 degrees for one hour. Dewaxing was done using two 15 minute xylene washes. The slides were then rehydrated using a series of 5 minute washes in 100%, 90%, 70% Isopropyl alcohol and TRIS buffer (pH – 7.4). Antigen retrieval was done using pressure cooker method in TRIS EDTA solution (pH – 9.2). Endogenous peroxidase was blocked using 0.3% hydrogen peroxide for 10 minutes. Primary rabbit monoclonal anti Survivin antibody (Path'nSitu, PR072) was applied and incubated at room temperature for 45 minutes followed by application of super enhancer for 15 minutes and secondary antibody tagged with HRP for 15 minutes (with 5 minutes TRIS buffer wash, two changes, in between each step). The reaction was viewed using diamino benzidine(DAB) as chromogen and haematoxylin counter stain.

Interpretation and scoring:

Scoring was done in a blinded fashion The mean percentage of tumor cells positive for Survivin expression was determined by examining 5 fields with highest expression, at 400 fold magnification.

Statistical analysis and results :

IBM SPSS version 21 was used for statistical analysis. The association between the explanatory and outcome variables was done by comparing the mean Survivin values across the groups. The mean differences and their 95% CI were presented. ANOVA was used to assess the statistical significance of the association. P value of less than 0.001 was considered significant.

Final diagnosis	Mean Survivin	Mean difference	P value	95% CI	
				Lower	Upper
Normal	0.010±0.03				
Adenoma	19.98±8.80	-19.97	<0.001	-25.60	-14.34
Adenocarcinoma	40.41±12.72	-40.40	<0.001	-46.03	-34.77

Mean Survivin expression was 0.01 in normal colonic mucosa, 19.98 in adenomas and 40.41 in adenocarcinomas, the difference was statistically significant.

Mean Survivin expression was 27.59 in well differentiated adenocarcinomas, 40.59 in moderately differentiated adenocarcinomas and 56.18 in poorly differentiated adenocarcinomas, the difference between each was statistically significant (P value less than 0.001).

S.No.	Clinico-Pathological parameter		N	Survivin expression	Mean difference	P
1	Age	<40	9	14.17±10.96	6.62	0.319
		>40	81	20.79±19.40		
2	Gender	Male	54	19.92	0.52	0.898
		Female	36	20.45		

3	Tumor grade	Well differentiated	10	27.59±4.43		
		Moderately differentiated	12	40.59±5.23	-13.00	<0.001
		Poorly differentiated	8	56.18±9.12	28.59	<0.001
4	Tumor stage	Stages I and II	21	37.66±10.38	9.18	0.069
		Stages III and IV	9	46.84±15.84		

DISCUSSION :

Evasion of apoptosis is an important step in carcinogenesis. Dysregulation of apoptosis can occur by down regulation of pro apoptotic factors or an over expression of anti apoptotic factors. This confers increased longevity to the cell and makes it prone to accumulate transforming mutations. Thus dysregulated apoptosis is involved in various stages of cancer including the emergence of tumor, increased survival and growth of the tumor, evolution of an aggressive clone, metastasis and has also been shown to confer tumor resistance to anticancer therapy.

There are three important antiapoptotic family of proteins which include FLICE-inhibitory proteins (FLIPs), Bcl-2 family and Inhibitors of Apoptosis Proteins (IAPs). The Inhibitors of Apoptosis (IAP) family of proteins are a group of proteins which inhibit the intrinsic pathway of apoptosis. . IAP has nine family members which are X-linked IAP, cIAP1, cIAP2, neuronal apoptosis inhibitor protein, melanoma IAP, IAP-like protein 2, livin, apollon, and survivin. Common to all the members of this family is the presence of Baculovirus IAP repeats (BIR), a 70 amino acid motif, in one to three copies which is essential for their function.

Survivin the smallest member of the IAP family of proteins is a 142 aminoacid containing 16.5 kDa protein.[5] It is encoded by the BIRC5 gene located at the telomeric position on chromosome 17q25.[6] Two main functions of Survivin are inhibition of apoptosis and regulation of cell division. Unlike other members of the IAP family, which bind to and promote the degradation of active initiator and executioner caspases, Survivin lacks the structural motifs necessary for binding caspases. It has a more complex mechanism of action. It functions by targeting the multi molecular processes involved in caspase 9 activation, in cooperation with other molecules like Hepatitis B X-interacting protein, X-linked IAP and by binding to and inhibiting smac/DIABLO, a proapoptotic protein.[7,8] Survivin is essential for mitosis and cytokinesis. It has a transcriptionally controlled expression at the G2/M phase and functions during a narrow window of time. With its expression during mitosis, survivin localises to various components of the mitotic apparatus including centrosomes, microtubules of metaphase, anaphase spindle and remnants of the mitotic apparatus suggesting that Survivin has an important role in microtubule dynamics and maintenance of normal bipolar mitotic apparatus.[9,10]

What makes Survivin clinically intriguing is its differential distribution in cancers compared to its limited expression in normal, terminally differentiated tissues. Survivin is normally expressed in embryonic and fetal tissues but is undetectable in terminally differentiated normal adult tissues. In contrast most human cancers have been shown to overexpress Survivin. Genome-wide searches have confirmed the differential expression of Survivin in tumors compared to normal tissues. The overexpression of Survivin has been shown to be consistently associated with more aggressive tumors, increased rates of recurrence, resistance to therapy and poorer prognosis than tumors that are negative for Survivin.[4,5]

The mechanisms by which survivin expression is deregulated in cancers include amplification of Survivin locus on chromosome 17q25, demethylation of survivin exons, increased promoter activity and increased upstream signalling in the PI3-kinase or MAP kinase pathways.[11,12,13] In addition, upregulation of Survivin expression in cancers is cell cycle independent, unlike normal cells.

The role of Survivin in cancers is much more than simple inhibition of apoptosis. Its dysregulation causes abnormality in mitotic spindle formation resulting in multiple genetic defects in the affected cells and a pro-mutagenic state and such cells are not eliminated by apoptosis. In addition to its role in tumorigenesis, malignant transformation and tumor progression, Survivin is also implicated in tumor angiogenesis and resistance to anti cancer therapy.[14,15]

In our study it was found that Survivin expression was practically absent in normal colorectal mucosa. Survivin localized to the nucleus of the cells of adenomas and adenocarcinomas and its expression increased from normal mucosa to adenomas and was maximally expressed in adenocarcinomas.The differences in mean Survivin expression between normal mucosa and adenomas and between adenomas and adenocarcinomas was statistically significant (P value less than 0.001). This result of the present study correlates with that of Hiroshi Kawasaki et al who in their 2001 study of colorectal neoplasia noted that the immunoreactivity of Survivin significantly increased from hyperplastic polyps to adenomas with low grade dysplasia and adenomas with high grade dysplasia and carcinomas which showed that survivin played an important role in the malignant transformation of adenomas.[16] Similar findings were made by Lian-Jie Lin et al who in their 2003 study inferred that the positive rate of survivin expression increased in transition from normal epithelium to adenoma with low grade dysplasia to adenoma with high grade dysplasia and carcinoma concluding that survivin expression is related with the early stage of colorectal carcinogenesis and plays an important role in the adenoma-carcinoma sequence.[17]

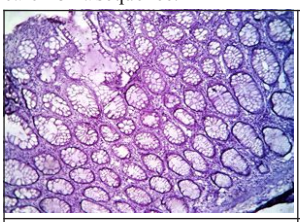

Fig 1.Normal colonic mucosa

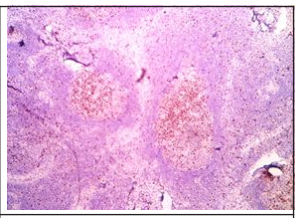

Fig 2. Survivin control –Lymph node.

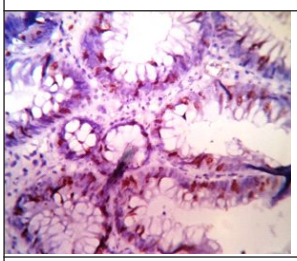

Fig 3.Survivin expression in well differentiated adenocarcinoma

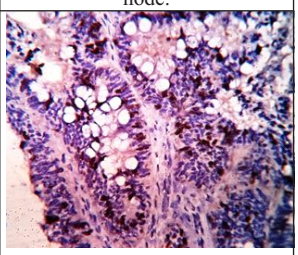

Fig 3.Survivin expression in moderately differentiated adenocarcinoma

Ulrike Gerlach et al in their 2006 study reported that Survivin expression correlated with the degree of differentiation of adenocarcinomas with similar conclusions drawn by the study of Hai Yan Tan et al.[18,19] The present study showed similar results with Survivin expression increasing from well differentiated to moderately differentiated adenocarcinomas with maximum expression seen in poorly differentiated adenocarcinomas. However there are other studies which have concluded that no such correlation could be demonstrated.[20,21,22]

In the present study no significant correlation was found between Survivin expression and patient age, gender and the stage of adenocarcinoma. Other studies have shown a poor survival with over expression of Survivin.[23,24]

CONCLUSION:

Survivin expression was negligible to absent in normal colonic epithelium with a significant increase in expression from adenomas to adenocarcinomas, suggesting that Survivin has an important role in early colorectal tumorigenesis and malignant transformation of adenomas(the adenoma-carcinoma sequence). This observation of minimal to absent Survivin expression in normal colonic epithelium and its significantly higher expression in adenomas and adenocarcinomas makes Survivin a potentially exploitable target of anti-cancer therapy with maximal targeting of the tumor and minimal damage to the normal epithelium. Survivin expression also showed a significant correlation with the degree of differentiation of adenocarcinomas. The results showed that dysregulation and over expression of Survivin expression is associated with an aggressive behaviour in colorectal adenocarcinomas. Detection of Survivin expression by immunohistochemistry may be used as a prognostic marker to predict tumor behavior.

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