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Original Article

The significance of sonic hedgehog immunohistochemical expression in colorectal carcinoma

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ABSTRACT

Colorectal carcinoma is a significant source of major morbidity and mortality. Sonic hedgehog (Shh) is expressed in normal gastrointestinal tract mucosa and in many malignancies. The purpose of the present study is to investigate the relationship between Shh immunoexpression in CRC and clinicopathological characteristics. Paraffin blocks of 155 primary CRCs and 37 nodal metastases were retrieved and tissue microarrays were constructed. Immunohistochemistry was performed using anti-Shh antibody. Immunostaining was scored and results were analysed in relation to the clinicopathological parameters. Shh was overexpressed in primary CRC ($p = 0.02$) and in nodal metastasis ($p = 0.004$). There was no difference between Shh immunoexpression in primary CRC and in nodal metastasis ($p = 0.941$). High Shh immunoexpression was associated with well differentiated tumours ($p = 0.004$). However, there was no association with other clinicopathological parameters. Shh overexpression was not associated disease free survival (log-rank = 0.079, $p = 0.778$). Shh is overexpressed in well differentiated CRC. However, Shh is not associated with other clinicopathological and prognostic factors. Loss of Shh may be associated with proliferation and loss of differentiation in CRC. Further molecular studies are required to address the potential importance of Shh signalling in CRC and to test Shh inhibitors and activators as potential therapeutic targets in CRC.

1. Introduction

Colorectal carcinoma (CRC) incidence and mortality rates remain one of the highest among other types of cancer worldwide [1]. Nodal metastasis and tumour stage remain the most important prognostic factors that the treatment plan is decided upon [2]. The pathogenesis of CRC involves a sequential process of genetic and molecular alterations that enhance cellular proliferation and suppress apoptosis [1,3]. It is essential to analyse these steps and correlate them with embryonic basis in order to identify other prognostic factors and modulate new targeted molecular interventions.

Cellular differentiation and proliferation during embryogenesis is regulated through the hedgehog (Hh)

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Table 1
Clinicopathological parameters of cases ($n = 155$).

Parameter		Number (%)
Sex	Male	78 (50.3%)
	Female	77 (49.7%)
Grade	Well differentiated	36 (23.2%)
	Moderately differentiated	98 (63.2%)
	Poorly differentiated	21 (13.5%)
Age	<60 years	88 (56.8%)
	≥60 years	67 (43.2%)
Tumour location	Right colon	41 (26.5%)
	Left colon	98 (63.2%)
	Rectum	16 (10.3%)
Tumour size	<5 cm	68 (43.9%)
	≥5 cm	87 (56.1%)
Primary tumour	T1	3 (1.9%)
	T2	22 (14.2%)
	T3	116 (74.8%)
	T4	14 (9%)
Nodal metastasis	Negative	80 (51.6%)
	Positive	68 (43.9%)
	Cannot be assessed	7 (4.5%)
Distant metastasis	Negative	111 (71.6%)
	Positive	44 (28.4%)
Lymphovascular invasion	Negative	132 (85.2%)
	Positive	23 (14.8%)
Margin status	Free	149 (96.1%)
	Involved	6 (3.9%)
Disease relapse	Negative	98 (63.2%)
	Positive	57 (36.8%)
Survival	Alive	94 (60.6%)
	Dead	26 (16.8%)
	Not available	35 (22.6%)

T1: tumour invades submucosa; T2: tumour invades muscularis propria; T3: tumour invades through the muscularis propria into the subserosa or into non-peritonealised pericolic or perirectal tissues; T4: tumour directly invades other organs or structures, and/or perforates visceral peritoneum.

signalling pathway [2]. Three members of the mammalian hedgehog were identified, Desert hedgehog (Dhh), Indian hedgehog (Ihh), and Sonic hedgehog (Shh) [4]. Shh expression was reported in normal gastrointestinal tract [2,5]. Continuous Shh signalling activity had been documented in multiple cancers, such as basal cell carcinoma, breast cancer, gastric cancer, pancreatic cancer, and CRC [5,6]. The activity of Shh signalling results in activation and nuclear translocation of the Gli family of transcription factors through multiple intracellular events. These transcription factors control the transcription of hedgehog target genes [7,8].

The aim of the present study is identify the significance of Shh immunoexpression in CRC in relation to the clinicopathological entities and patient outcome.

2. Materials and methods

2.1. Patients

The study included paraffin wax blocks of primary tumour from 155 patients with CRC and corresponding 37 nodal metastases. Blocks were retrieved from the archives of the Department of Pathology at King Abdulaziz University, Jeddah, Saudi Arabia. Clinicopathological characteristics of patients are listed in Table 1. The study was approved by the Research Committee of the Biomedical Ethics Unit, Faculty of Medicine, King Abdulaziz University.

2.2. Tissue microarray

Tissue microarrays (TMA) were designed and constructed as previously described [9]. Haematoxylin and eosin-stained sections of primary tumours and nodal metastasis were reviewed by an experienced pathologist (WG). Areas of interest were chosen from the original blocks and were marked on the slides. Necrotic areas, autolytic areas and areas containing predominantly the stromal tissue were avoided. Primary CRC and nodal metastasis paraffin-embedded blocks were retrieved and examined for validity to perform TMA. Two tissue cores each 1.5 mm in diameter were punched from each donor block in an automated TMA instrument (TMA Master 1.14 SP3 from 3D Histech Ltd., Budapest, Hungary) and inserted into a recipient paraffin block. Placental tissue was used for orientation. Slides were cut from TMA block and stained with haematoxylin and eosin for initial morphological assessment of accuracy of construction.

2.3. Immunohistochemistry

TMA blocks were cut at 4 μm, and mounted on positive-charged slides (Leica Microsystems Plus Slides). Sections were deparaffinised in xylene and rehydrated in an automated immunostainer (BenchMark XT, Ventana® Medical systems Inc., Tucson, AZ, USA). Pre-treatment was done using CC1 (prediluted cell conditioning solution) for 60 min. Anti-human rabbit anti-Shh polyclonal antibody (Spring™ Bioscience; Cat # E17920) was incubated at 37 °C for 20 min. Ventana® I-view DAB detection kit was used according to kit manufacturer instructions. Subsequently, slides were washed, counterstained with Mayer's haematoxylin and mounted. Negative control (substitution of the primary antibody with Tris–buffered saline) and positive control slides were included.

2.4. Interpretation of Shh immunostaining

Sections were evaluated independently without knowledge of the clinicopathological characteristics of patients. The expression pattern was determined independently by 3 investigators (DG, WG, and AA). Cytoplasmic staining of tumour cells was evaluated. Observer's bias was avoided by repeating the evaluation of protein expression at 2 different time points and without knowledge of patients' clinical data. Both staining intensity and extent (percentage) were noted. The percentage was calculated by counting the percentage of positive tumour cells within the total number of tumour cells in sections. The percentage was expressed as; (1) when 0–10% of malignant cells were positive, (2) when 11–50% of malignant cells were positive, and (3) when labelling in more than 50% of malignant cells. The staining intensity was reported as; (0) negative; (1) weak; (2) moderate; and (3) high. For statistical purpose, a combination was done between intensity and percentage and was given a numerical 6-scale score. Results were finally dichotomised as low expression when score was 1–3 and high expression when score was 4–6.