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Original Contribution

Aberration of Nrf2-Bach1 pathway in colorectal carcinoma; role in carcinogenesis and tumor progression

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ABSTRACT

Nrf2 and Bach1 are important transcriptional factors that protect against reactive oxygen species (ROS). Although aberration of these molecules was associated with malignant transformation and progression, their aberration pattern in colorectal carcinoma (CRC) is not yet fully studied.

In this study, Nrf2 and Bach1 were immunohistochemically examined in 93 formalin-fixed paraffin-embedded blocks of colonic tumors (65 carcinoma with their corresponding surgical margins and 28 adenomas).

Nrf2 expression was gradually increased in the apparently normal mucosa (57 ± 41)-adenoma (90 ± 36)-carcinoma (198 ± 78) direction and only showed significant higher mean of expression in CRC with brisk inflammatory peritumoral response. The mean of Bach1 expression was highest in apparently normal colonic mucosa (267 ± 16), lowest in adenoma (53 ± 31) and high in carcinoma tissues (194 ± 93). Significant higher mean of expression was detected in carcinoma with: LN metastasis ($p = 0.04$), lymphovascular invasion ($p = 0.024$); perineural invasion ($p = 0.03$) and advanced pathological stage ($p < 0.001$). Significant higher mean of expression of Nrf2 and Bach1 was detected in adenoma specimens with high grade dysplasia ($p = 0.016$ and $p = 0.024$) respectively.

In conclusion, Nfr2 and Bach1 expression are altered in CRC but in different way. Nrf2 is gradually increasing from normal mucosa to adenoma and was highest in carcinoma but was not associated with features of tumor invasiveness. Bach1 was highest in normal mucosa; less in adenoma then increased in carcinoma and was associated with features of tumor invasiveness and metastasis. This may indicate a possible role of Nrf2 in CRC carcinogenesis and a role of Bach1 in CRC progression.

1. Introduction

Colorectal carcinoma (CRC) is one of the leading cancer-related deaths all over the world. In spite of the great advances in diagnosis and in developing therapeutic options, the disease is still a challenging problem [1]. During the last two decades, most of the molecular basics of CRC have been identified. The main pathway through which most CRC emerge is the adenoma-carcinoma pathway. In this pathway, the normal colonic mucosa underwent several sequential molecular changes upon exposure to carcinogenic factors over years. These changes start with mutation in one of the major cellular regulating genes; oncogenes, tumor suppressor genes, apoptosis-regulated genes and mismatch repair genes followed by further mutation in other genes ending by malignant transformation. Two main genetic pathways have been described in carcinogenesis of CRC; the first is the *APC/Wnt* pathway where mutation starts with *APC* gene followed by *RAS* and *P53* genes and the other pathway is the MMR (mismatch repair) pathway which involves mutation in *MSH* and *MLH* genes [2]. These changes are now appreciated to develop gradually over years in a stepwise manner known as the multistep theory of carcinogenesis.

One of the novel genetic changes that has been suggested to play a key role in some types of malignant tumors is the Nrf2-Bach1 pathway. Nrf2 is the “nuclear factor erythroid 2 p45-related factor 2” which is a transcriptional factor produced ubiquitously in the cellular cytoplasm where it functions as a key regulator for the redox balance inside the cell.

During cellular metabolism and as a byproduct of oxidative phosphorylation process, reactive oxygen species (ROS) are produced. ROS molecules should be tightly regulated within the cell to be maintained in a certain balance called the “redox balance”. Imbalance or excess of ROS has been found to be a causing factor for many diseases including inflammation and cancer formation. Nrf2-Bach1 pathway is in the heart of the regulating network of this balance [3].

In normal conditions, Nrf2 is produced in the cytoplasm then

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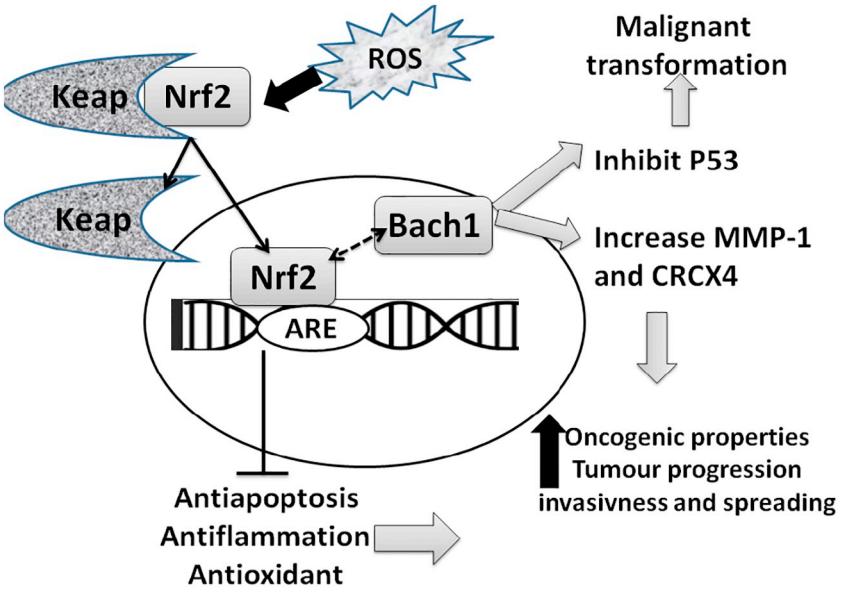

Fig. 1. Cellular effect of Nrf2 and Bach1.
Nrf2 is produced in the cytoplasm, it is rapidly bounded and degraded by keap protein. On exposure to ROS, Nrf2 is released from keap and is transported to the nucleus where it binds antioxidant related elements (ARE) to increase transcription of enzymes that counteract the ROS effect. Bach1 competes with Nrf2 at the DNA binding sites. It also inhibits P53 allowing mutation to be fixed causing malignant transformation. On the other side, Bach1 also induce transcription of genes MMP-1and CXCR4 which induce invasiveness of tumor cells.

rapidly degraded by ubiquitation through binding to the "Keap" molecule. Upon exposure to high levels of ROS, Nrf2 is separated from Keap and is translocated to the nucleus. In the nucleus, Nrf2 stimulates transcription of the antioxidant related element (ARE) genes which encode antioxidant enzymes that counterbalances the excess of ROS within the cell bringing the Redox balance to normal (Fig. 1) [3]. Malfunction of Nrf2 would allow ROS to accumulate and cause cellular damage.

Nrf2 was found also to be implicated in controlling and regulating about 600 genes. These genes encodes proteins and enzymes that are involved in several aspects in cytoprotection including; removal of damaged proteins, protection against inflammation [4,5], protection against heavy metals, growth factors and transcription factors [6]. The end result of Nrf2 activation is protection of the cell against inflammatory agents, toxic factors and carcinogenic factors [7-11]. No wonder, malfunction of Nrf2 was reported in several disorders. Nrf2 was found malfunctioning in neurological cells derived from patients with neurological disorders as Parkinson's disease, in lung cells from patients with pulmonary emphysema [12] and lately was described in malignant transformation [13].

BACH1 (BTB Domain and CNC Homolog 1) is a protein coding gene that was classified to function as a transcriptional factor. It was found to be essential to keep the redox balance normal within the cell. Its cytoplasmic regulation and nuclear localization is not well understood as for Nrf2. Bach1 is not working as a downstream molecule for Nrf2 but they both work to keep the redox balance normal. It was found that Nrf2 functions are down-regulated by Bach1 [14] and in fact that Bach1 competes against Nrf2 for the ARE gene promoter site inside the nucleus (Fig. 1) [15].

In an interesting recent bioinformatics analysis of transcriptional microarray data, Bach1 has emerged as a key player in cancer progression [16]. Bach1 was found in the analysis to be associated particularly with increase in transcription of metastasis-deriving genes such as matrix metalloproteinase and chemokine receptors [16]. This was further validated by experimental analysis where Bach1 overexpression was found to promote the migration and invasion of cancer cells, whereas its knockdown significantly suppressed these processes [16].

These findings shed light on the possible important role of Nrf2-Bach1 pathway in colorectal neoplasia, but it is not known if they play that role in early stages of carcinogenesis or during progression of malignant tumors. Few studies have examined the aberration in Nrf2-Bach1 pathway in human tissues and in colorectal neoplastic tissue in particular. Aims of the following study were: (1) to assess expression of both Nrf2 and Bach1 protein molecules in CRC tissues and their pre-neoplastic lesion (colorectal adenoma) and comparing this expression with normal colonic mucosa (2) to correlate between aberration in Nrf2-Bach1 expression and the clinicopathological criteria of CRC specimens including presence of lymph node metastasis and pathological stage of tumors.

2. Material and methods

2.1. Specimens

The study was conducted retrospectively on 93 specimens of colorectal neoplastic tissues; 65 primary CRC and 28 adenomas. Tissues were formalin-fixed paraffin embedded blocks obtained from the archive of the surgical pathology lab, Assiut University Hospital, Faculty of Medicine, Assiut University. In each of the CRC specimens, three tissues were examined immunohistochemically; 1- the tumor tissues, 2- tumor overlying mucosa, 3- apparently normal mucosa at surgical margin (away from the primary tumor site by at least 6 cm). All specimens included in this study were non-selected consecutive series of colorectal carcinoma and colonic adenomas referred to the lab. The available clinicopathological features were retrieved from the hospital medical records, including patient age, gender, tumor site, tumor size and type of operation.

Hematoxylin and eosin stained sections of colorectal carcinoma were examined for detailed histopathologic features including histologic type (according to WHO histologic classification 2010) [17], histopathological grade, host immune response, tumor stage [according to AJCC Cancer Staging Handbook of the American Joint Committee on

Cancer [18]], the presence or absence of lymphovascular emboli and the presence or absence of perineural invasion. Also, adenoma specimens were examined and classified according to the degree of dysplasia into low and high grade (Table 1).

2.2. Immunohistochemical staining

Immunohistochemical staining was performed using the avidin–biotin immunoperoxidase method. Tissue sections of 4 μm thickness of formalin-fixed paraffin-embedded specimens were taken from tissue blocks. Sections were dewaxed and then rehydrated through descending graded ethanol series, down to distilled water. To block the endogenous peroxidase, the rehydrated sections were treated with 6% hydrogen peroxide for 7 min. For epitope retrieval, sections were microwaved in citrate buffer, pH 6 for a total 20 min. Nonspecific staining had been blocked by superblock (UV block) for 10 min. Sections were incubated with the primary antibodies for 120 min. The antibodies used was Bach-1 (clone 4E11, Novus biologicals, diluted at1/150) and Nrf2 (clone N/A, Spring bioscience, diluted at 1/150). Secondary staining kits were used according to the manufacturer's instructions (Thermo scientific corporation Fremont, CA, USA). Counter staining was done with hematoxylin and examined by light microscopy.

2.3. Evaluation of Bach-1 and Nrf-2 expression

Both Bach-1 and Nrf2 were assessed semiquantitavely using IHC (H score); (IHC score was calculated by multiplying degree of staining “0, 1, 2, 3” by the percentage of positive staining“ 0–100 %”) to give a maximum histoscore of 300. Nrf2 showed cytoplasmic and nuclear staining. The frequency histograme of the Nrf2 values showed a trough at the 0 value, specimens were therefore categorized into two categories; specimens with positive Nrf2 expression (those > 0 expression) and specimens with negative Nrf2 expression (those < 0 expression), the same method of assessment was used in previous studies [19]. Bach1 showed only nuclear reactivity, its expression was considered positive if score > 0.

2.4. Statistical analysis

Mann-Whitney test and Kruskalwallis (K-test) were used to compare the means of Bach-1 and Nrf2 expression in the studied cases in relation to different clinicopathological features. Also, both tests were used to compare the mean of expression of these markers in colorectal carcinoma, overlying mucosa, surgical margins and adenoma specimens. *P* values of < 0.05 were regarded as statistically significant. Spearman correlation coefficient was used to investigate the correlation between Bach-1 and Nrf2 expression. *P* values of < 0.05 were regarded as statistically significant.

3. Results

3.1. Clinicopathological characteristics

The patients' clinicopathological characteristics are summarized in (Table 1). Briefly, the 65 evaluated cases of colorectal carcinoma include; 52 adenocarcinoma, 7 mucoid carcinoma and 6 cases signet ring carcinoma. The mean of age of the patients at the time of diagnosis was 48.94 ± 14.8.

Table 1
Clinicopathological features of the studied cases.

Clinicopathological features	Number	Percentage
Colorectal carcinoma		
Total	65	100%
Age		
≤50	36	55.4%
> 50	29	44.6%
Gender		
Men	25	38.5%
Women	40	61.5%
Tumor size (cm)		
Median	5.00	
Range	2–12	
Localization of the tumor		
Rectum	12	18.5%
Rectosigmoid	6	9.2%
Sigmoid colon	14	21.5
Descending colon	8	12.3%
Left flexure	2	3.1%
Transverse colon	4	6.2%
Right flexure	4	6.2%
Ascending colon	4	6.2%
Cecum	11	16.9%
Localization (grouped)		
Left colon	44	67.7%
Right colon	21	32.3%
Histopathological type		
Adenocarcinoma	52	80%
Mucoid carcinoma	7	10.8%
Signet ring carcinoma	6	9.2%
Grade of adenocarcinoma		
Low grade	47	90.4%
High grade	5	9.6%
T stage		
T1	1	1.5%
T2	14	21.5%
T3	29	44.6%
T4	21	32.3%
N stage		
Nx	8	12.3%
N0	26	40%
N1	13	20%
N2	18	27.7%
N stage (grouped)		
Nx	8	12.3%
N positive	31	47.7%
N negative	26	40%
Lymphvascular invasion		
Positive	38	58.5%
Negative	27	41.5%
Perineural invasion		
Positive	8	12.3
Negative	57	87.7%
Host immune response		
Mild	29	44.6%
Moderate	22	33.8%
Brisk	14	21.5%
Adenoma		
Age (years)		
Mean of age	31.64 ± 8.1	
Gender		
Men	19	67.8%
Women	9	32.1%
Adenoma type		
Low grade dysplasia	15	53.5%
High grade dysplasia	13	46.4. %

For the 28 adenoma cases, the mean of age of patients was 31.64 ± 8.1. Fifteen cases showed low grade dysplasia and the remaining thirteen cases showed severe form of dysplasia (Table 1).

3.2. Expression of Bach1 and Nrf2

Bach1 and Nrf2 expression were investigated in 65 CRC and 28 adenoma specimens. Bach1 showed positive nuclear staining pattern while the expression of Nrf2 was both nuclear and cytoplasmic. Both markers showed variable staining reactivity for the tumor inflammatory cells.

Frequency of Bach1 expression: Bach1 was positively expressed in 59/65 colorectal carcinoma specimens (90.7%), in all tumor-overlying mucosa 65/65(100%) and in all apparently normal mucosa at surgical margins 65/65 (100%). In adenoma, 22/28 specimens (78.6%) showed positive expression (Table 2& Fig. 2). *Degree of expression:* Bach1 showed highest mean of expression in apparently normal mucosa at surgical margins followed by carcinoma, then overlying tumor mucosa and was least in the adenoma group ($p < 0.001$).

The mean of Bach1 expression was significantly higher in carcinoma comparing to adenoma, overlying mucosa with ($p < 0.001$, $p = 0.003$) respectively. On the other hand, the mean of Bach1 expression was significantly lower in carcinoma comparing to normal mucosa at surgical margin ($p < 0.001$) (Table 4).

Nrf2 frequency of expression: Positive staining for Nrf2 was detected in 60/65(92.3%) of CRC specimens, 58/65(89.2%) of overlying mucosa, 46/65(70.7%) of the apparently normal mucosa at surgical margins and in 25/28(89.3%) of adenoma specimens (Table 2 & Fig. 3). *Nrf2 degree of expression:* A gradual increase in the mean of Nrf2 expression was seen from normal mucosa at surgical margin to adenoma to tumor-overlying mucosa to carcinoma with ($p < 0.001$). A significant higher mean of expression of Nrf2 was detected in carcinoma comparing to adenoma, tumor-overlying mucosa and normal mucosa groups with ($p < 0.001$) for each (Table 4). Expression of Nrf2 in adenoma was significantly higher if compared to normal mucosa group with ($p = 0.001$) (Table 4).

Table 2
Frequency of expression of Bach1 and Nrf2 in different groups (colorectal carcinoma, overlying mucosa, surgical margins and adenoma).

Specimen type	Bach1			Nrf2		
	No	Positive	Negative	No	Positive	Negative
Colorectal carcinoma	65	59(90.7%)	6(9.2%)	65	60(92.3%)	5(7.6%)
Overlying mucosa	65	65(100%)	0	65	58(89.2)	7(10.7)
Surgical margins	65	65(100%)	0	65	46(70.7%)	19(29.2%)
Adenomas	28	22(78.6%)	6(21.4%)	28	25(89.3%)	3(10.7%)

3.3. Association between expression of Nrf2 and Bach1 with clinicopathological criteria in colorectal carcinoma and adenoma

The mean of Bach1 expression was significantly higher in tumors with lymph node metastasis ($p = 0.040$), positive lymphovascular emboli ($p = 0.024$), positive perineural invasion ($p = 0.030$) and with more invasive tumors ($p < 0.001$). No significant difference in the mean was detected regarding; patient age ($p = 0.392$), gender ($p = 0.498$), tumor size ($p = 0.440$), location of the tumor ($p = 0.463$), histopathological type of the tumor ($p = 0.404$), tumor grade ($p = 0.318$) or with host immune response ($p = 0.061$).

In adenoma specimens, higher mean of Bach1 expression was significantly associated with adenomas with high degree of dysplasia ($p = 0.024$) (Table 3).

For Nrf2, the only significant difference was detected between high Nrf2 expression in colorectal carcinoma and host immune response ($p < 0.001$). However, no significant difference was found between degree of Nrf2 expression and patient age, gender, tumor size, location, histopathological type, grade, depth of tumor invasion, presence of lymph nodal metastasis, presence of lymphovascular emboli or presence of perineural invasion ($p = 0.454$, $p = 0.161$, $p = 0.061$, $p = 0.949$, $p = 0.236$, $p = 0.767$, $p = 0.466$, $p = 0.872$, $p = 0.322$ and $p = 0.160$) respectively (Table 3).

In adenoma specimens, significant higher mean of expression of Nrf2 was associated with high degree of dysplasia ($p = 0.016$) (Table 3).

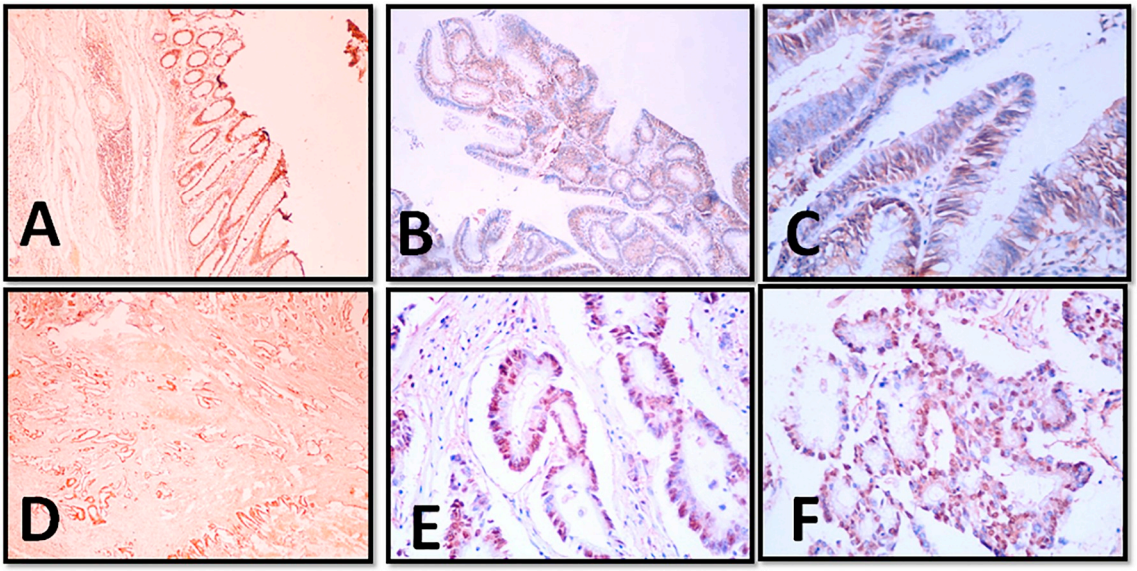

Fig. 2. Expression of Bach-1 in colorectal carcinoma, adenoma and normal mucosa.
A) Normal mucosa at the surgical margins showing intense positive expression of Bach1 in the mucosal cells and in the perivascular lymphocytic aggregates around the blood vessels in submucosa x100. B) Adenoma tissue showing lower degree of nuclear expression x100. C) The same specimen in image B at higher magnification x400. D) Colorectal carcinoma tissue showing highest degree of Bach-1 expression x40. E) and F) A high magnification of two other examples of CRC tissue showing high degree of Bach-1 positive staining x400 and x400 respectively.

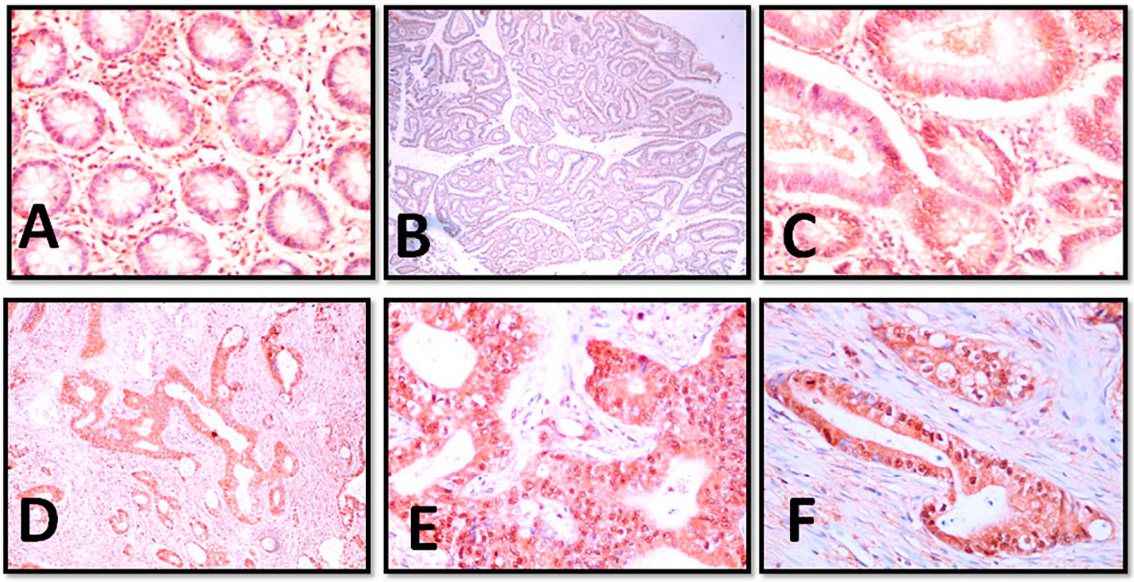

Fig. 3. Expression of Nrf-2 in colorectal carcinoma, adenoma and normal mucosa.
A) Normal mucosa at the surgical margins showing relatively weak positive expression of Nrf2 in the mucosal cells x400. B) Adenoma tissue showing moderate expression of Nrf2 x100. C) The same specimen in image B at higher magnification x400. D) Colorectal carcinoma tissue showing strong Nrf2 expression x100. E) and F) The same specimen in image D at higher magnification x400 and x400 respectively.

4. Discussion

The significantly different geographic incidence of CRC among the world countries, with the highest rates reported in the developed industrialized countries, made it clear that the environmental carcinogenic factors are to be blamed for the carcinogenesis of CRC [1]. The age incidence of CRC varies worldwide with most of the cases reported in those aged 50 years or older. Our study found higher incidence among patients < 55 years. Recent studies from Egypt [20] and from other countries described a recent higher incidence of CRC among lower age group [21]. This may be due to environmental exposure to certain carcinogenic factors among younger generation especially after acquisition of westernized life style that depends on fast high fat-containing meals.

Because many of the carcinogenic factors exert their mutagenic effects through release of excess ROS, a recent interest has been directed towards Nrf2-Bach1 pathway in neoplasia formation. The following study assessed expression of Nrf2 and Bach1 in colorectal carcinoma, apparently normal mucosa and adenoma tissues.

Nrf2 has been reported to play a significant role in protecting the cell against carcinogenic factors through upregulation of antioxidant enzymes to get rid of any excess ROS within the cell. Nrf2 is then expected to be lost during transition from normal to adenoma to carcinoma tissues; however the opposite was detected in the specimens studied here. Nrf2 expression showed a gradual increase from normal colonic mucosa at surgical margins, to adenoma tissues and was highest in the CRC tissues. These results were in consistent with those reported by similar studies [22], however against findings from others [23].

Compelling evidence from studies has started to shape Nrf2 as a double-edged sword. On one side, Nrf2 resists malignant transformation by keeping the “Redox balance” at normal level. On the other side and after malignant transformation, aberrant high expression of Nrf2 was found to induce transcription of the antiapoptotic proteins Bcl-2 and Bcl-xL within the cells increasing their ability to resist apoptosis [13]. Even worse, dysregulation of Nrf2 within the tumor cells gives them a prosurvival phenotype *i.e.* they acquire sustained ability for tumor growth and resistance to oxidants and anticancer drugs [14] which ends up by having a chemoresistant tumor [24]. Aberration of the Nrf2 expression was found to be associated with poor response to therapy in some malignant tumor types [25]. Modulation of the Nrf2 pathway was then suggested to have therapeutic potential [26].

We did not find significant relationship between aberrant high expression of Nrf2 with any of the known clinicopathological criteria of CRC apart from association with brisk inflammatory immune cellular response. This is unlike previous studies which reported that high Nrf2 expression was correlated with the presence of LN and distant metastasis [27]. A recent study reported a significant relationship between tumor infiltrating immune cells and Nrf-2 [28]. In this study, expression of Nrf-2 was found up regulated in tumor infiltrating T lymphocytes (TILs). This upregulation was explained by the hypoxic condition within the tumor environment.

The pattern of Bach1 expression in the colonic tissues studied here is different from Nfr2. Bach1 did not show that gradual increase in expression in the normal-adenoma-carcinoma direction. Its expression was highest in the normal mucosa at surgical margins then it drops in adenoma and increased again in carcinoma and its overlying mucosa.

In literature, the role of Bach1 in early carcinogenesis is controversial. It was postulated that Bach1 may not be essential for early carcinogenesis but after tumor formation, aberration of Bach1 expression is expected to result in tumors that resist chemotherapeutic agents. Upon exposure to oxidative stress, p53 stimulate cell senescence or death to prevent fixation of DNA damage or to pass this damage to next cell generations. Bach1 prevents cellular senescence by formation of a binding complex with P53.

In 2011 a study analyzed human gene expression data in relationship to prostate cancer progression hypothesized that Bach1 may be an important player in progression of prostate cancer [29] however this was not proven experimentally or examined on human tissue. A recent interesting study by Liang and colleagues examined transcriptome profile in breast cancer cell lines found that Bach1 is a metastasis master regulator gene. Bach1 overexpression promotes the migration and invasion of cancer cells, whereas when knockdown significantly suppressed these processes. This effect was found to be through reduction of matrix metalloproteinase-1 (MMP1) and the chemokine (CXCR4); key proteins in cell invasion and metastasis [16]. Bach1 was later grouped with the pro-metastatic genes and was subsequently suggested to be an important target for therapeutic intervention [30,31].

Table 3
Relationship between Bach1 and Nrf2 expression and the clinicopathological factors in colorectal carcinoma and adenoma.

Clinicopathological factors	Bach1		Nrf2	
	Mean	*p* value	Mean	*p* value
Colorectal carcinoma				
Age				
≤50	203.42 ± 91.7		206.42 ± 74.4	
> 50	184.14 ± 95.7	0.392	189.45 ± 84	0.454
Gender				
Men	185.68 ± 94.8	0.498	219.00 ± 71	0.161
Women	200.52 ± 93.1		186.25 ± 81.4	
Size				
≤5 cm	190.90 ± 91.5	0.440	183.27 ± 88.7	0.061
> 5 cm	201.50 ± 97.8		225.46 ± 48.8	
Localization (grouped)				
Left colon	197.70 ± 96.2	0.463	195.45 ± 81.2	0.949
Right colon	188.76 ± 88.8		205.95 ± 74.6	
Histopathological type				
Adenocarcinoma	192.27 ± 98		190.58 ± 81.2	
Mucoid carcinoma	172.86 ± 82.2	0.404	231.43 ± 67.5	0.236
Signet ring carcinoma	242.50 ± 41.8		232.50 ± 55.1	
Grade of adenocarcinoma				
Low grade	195.91 ± 97.3	0.318	190.74 ± 83.8	0.767
High grade	158.00 ± 109.6		189.00 ± 56.1	
T stage				
T1	85.00		210.00	
T2	117.14 ± 81.9	< ***0.001***	213.14 ± 83.9	0.466
T3	193.17 ± 98.5		204.00 ± 76.1	
T4	254.10 ± 37		181.67 ± 81.5	
N stage				
N positive	227.39 ± 76	***0.040***	200.65 ± 76.6	0.872
N negative	183.85 ± 91.5		190.38 ± 87	
Lymphvascular invasion				
Positive	219.32 ± 80.5	***0.024***	189.74 ± 83.6	0.322
Negative	160.33 ± 100.4		211.67 ± 70.8	
Perineural invasion				
Positive	254.62 ± 50.1	***0.030***	168.12 ± 89.6	0.160
Negative	186.42 ± 95.1		203.16 ± 76.9	
Hostimmune response				
Mild	187.31 ± 88.5		141.55 ± 76.5	
Moderate	178.27 ± 107.5	0.061	236.36 ± 42	< ***0.001***
Brisk	236.36 ± 69.6		258.57 ± 38.5	
Adenoma				
Gender				
Men	52.89 ± 34.8	0.842	83.16 ± 46.07	0.285
Women	53.89 ± 26.1		105.56 ± 36.09	
Type				
Low grade dysplasia	40.67 ± 32.23	***0.024***	70.0 ± 47.9	***0.016***
High grade dysplasia	67.69 ± 25.46		113.85 ± 22.56	

Table 4
relation between means of Bach-1 and Nrf2 expression and different groups (carcinoma, adenoma, overlying mucosa and normal mucosa at surgical margin).

	Bach1		Nrf2	
	Mean	*p* value	Mean	*p* value
Carcinoma *vs* Overlying mucosa	194.82 ± 93.3 163.15 ± 72.9	***0.003***	198.85 ± 78.7 109.66 ± 48.1	< ***0.001***
Carcinoma *vs* normal mucosa at surgical margin	194.82 ± 93.3 267.92 ± 16.5	< ***0.001***	198.85 ± 78.7 57.23 ± 41.9	< ***0.001***
Carcinoma *vs* adenoma	194.82 ± 93.3 53.2 ± 31.8	< ***0.001***	198.85 ± 78.7 90.36 ± 43.75	< ***0.001***
Overlying mucosa *vs* normal mucosa at surgical margin	163.15 ± 72.9 267.92 ± 16.5	< ***0.001***	109.66 ± 48.1 57.23 ± 41.9	< ***0.001***
Normal mucosa at surgical margin *vs* adenoma	267.92 ± 16.5 53.2 ± 31.8	< ***0.001***	57.23 ± 41.9 90.36 ± 43.75	***0.001***

The studies about Bach1 in human tissues are limited; in our study we found a significant relationship between high expression of Bach1 with all features of tumor invasiveness and metastatic features. High Bach1 expression was positively correlated with the presence of lymphovascular invasion, presence of neural invasion, higher clinicopathological stage and with the presence of LN metastasis. All our findings confirm the results reported by the aforementioned genes expression studies. Based on the findings from this study and what was reported in literature about the Nrf2-Bach1 pathway, part of the interaction network between Nrf2 and bach1 is illustrated in figure-1.

In conclusion, this study found that expression of both Nrf2 and Bach1 is altered in CRC but in different way. Nrf2 is gradually increased from normal mucosa to adenoma and was highest in carcinoma but was not associated with features of tumor invasiveness. The opposite was found with Bach1; the protein was highest in normal mucosa less in adenoma then increased in carcinoma and was associated with features of tumor invasiveness and metastasis. This may indicate a possible benefit from targeting Bach1 in targeted therapeutic agents in CRC.

Abbreviations

APC	adenomatous polyposis coli gene
ARE	antioxidant related element
Bach1	BTB Domain and CNC Homolog 1 gene
CRC	colorectal carcinoma
CXCR4	chemokine receptor 4
MMP-1	matrix metalloproteinase -1
MMR	mismatch repair gene
Nrf2	nuclear factor erythroid 2 p45-related factor 2
ROS	reactive oxygen species

Declaration of interest

None.

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