Immunohistochemical Expression of c-Myc and p53 Proteins in Colorectal Carcinoma

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Received ٢٢/١/2009 – Accepted ٢٢/٣/2010

ABSTRACT

The purpose of this study was to investigate the possible role of c-Myc and p53 in patients with colorectal cancer by using immunohistochemical analysis. The paraffin embedded sections from 40 colorectal carcinoma and 40 healthy individuals were investigated for the expression of c-Myc and p53 by immunohistochemical staining with specific monoclonal antibodies. Our data analysis demonstrated a significantly increased expression of c-Myc and P53 among colorectal cancer patients compared with control groups (p<0.01). In addition our results showed that in patients with colorectal cancer the positive expression rate was 80% and 62.5% for c-Myc and p53, respectively. Furthermore, in this study the significant correlations was found between these two markers (p<0.05) in two studied groups. Our results confirms a significant association between colorectal carcinoma and increased expression of c-Myc and P53.

INTRODUCTIONS

Colorectal cancer is the second most common cause of cancer-related mortality in Western countries, with about 1 million new cases every year diagnosed world-wide and 500,000 patients dying from the disease [1]. The steps to colorectal cancer are driven by genetic alterations in oncogenes and tumor suppressor genes [2]. The c-Myc oncogene has been shown to be amplified and/or overexpressed in many types of human cancer [3;4]. The role for c-Myc in the development of colon tumors was first suggested by the report that c-Myc was amplified and overexpressed in a chemically induced mouse colon tumor [5]. The central role of c-Myc protein in accelerating cell proliferation, documented by many early studies, has led to a general concept for
many types of cancer that amplification or overexpression of this gene may be associated with a more aggressive tumor and a poorer patient survival [4;6;7]. The overexpression of c-Myc protein has also been shown to associate with a better tumor differentiation or a better patient survival for cancer of the testis, ovary, bile ducts, colon and breast [8;9].

On the other hand, P53 is a tumor suppressor gene that plays a key role in the control of the cell cycle. Cell proliferation is inhibited by normal or wild type p53 protein, which acts by arresting the cell cycle at the G1-S phase to allow DNA repair to take place. Loss of this activity may lead to neoplastic transformation. Alteration of this suppressor gene is a common event in colorectal cancer and has been associated with adverse postoperative outcome and poor survival [10;11]. It is widely accepted that multiple genetic alterations underlie colorectal carcinogenesis. The p53 mutation is common in human cancers and overexpression of its products is detected in many colorectal cancers. Thus, the immunohistochemical detection of the overexpression of p53 is a useful marker for the diagnosis of carcinoma [12;13]. However, the relationship between the p53 overexpression and metastasis in colorectal cancers is still controversial [14;15].

The purpose of this study was to investigate the c-myc and p53, protein expression within the same colorectal cancer tissue by using immunohistochemistry technique, to find out the correlation between these two markers.

**MATERIAL AND METHODS**

**Patients:** This study included 80 patients from Baghdad Teaching Hospital, AL-Yarmook Teaching Hospital, Gastroenterology and Hepatology Teaching Hospital and private hospital. This study was carried out on (40) patients with colorectal carcinomas (CRC) (22 males and 18 females) with a mean of age 51.7 years and a range between 20 and 81 years. The control group included (40) colorectal normal tissue (CRN) (22 males and 18 females) with a mean of age 49.2 years and a range between 20 and 75 years.

**Samples:** For each patients and control included in this study; serial sections from paraffin embedded block were taken from the archive of department of pathology of these hospitals (mention above). Tissue sections cut into 5μm thickness, put on Fisher_ brand positively charged slides.

**Immunohistochemical analysis (IHC) for detection of c-Myc and p53 proteins expression in paraffin embedded sections:**

The use of universal DakoCytomation streptavidin- biotin system purchased from DakoCytomation (USA) Immuno-histochemistry detection kit. The mouse anti-human monoclonal antibodies p53 protein
The primary antibody reacts with antigen in the tissue, and then a biotin labeled secondary antibody (link antibody) binds to the primary antibody. When the conjugate is added, the biotinylated secondary antibody will form a complex with the peroxidase-conjugated streptavidin and by adding the substrate, which contains 3,3'-diaminobenzidine (DAB) in a chromogen solution, a brown-colored precipitate will form at the antigen site. In the peroxidase secondary detection system, the presence of a brown reaction product at the site of the target antigen is indicative of positive reactivity. Counter stain will be pale to dark blue coloration of the cell nuclei. Evaluation of the immunostaining was done with the assistance of a histopathologist. The observer was blinded to the clinical diagnosis of the tissues at the time of assessment, and tissues were independently assessed by two observers positive or negative cases, positive immunostaining gave nuclear and/or cytoplasmic dark brown granules. Counting the number of positive cells which gave brown cytoplasmic staining system under light microscope. The extent of the IHC signal was determined in 10 fields (X100 magnification). In each field the total number of cells was counted and the extent of cytoplasmic staining cells was determined as a percent. The total staining score was divided by the number of whole cells per field in 10 fields, so the percentage of positively stained cells in the 10 fields was calculated for each case by taking the mean of the percentage of the positively stained cell in the 10 fields. Specimens in which less than 10% of the cancer cells were immunostained with p53 were classified as negative, and the rest were classified as positive, as described by Jeng et al[16]. But cancer were regarded as c-Myc positive when their immunoreactivity scores were ≥ 1 . Cancer with immunoreactivity zero were regarded as c-Myc negative [17].

Statistical analysis: Student test (t-test) was used for the quantitative data. The relationship between the markers was measured qualitatively by using the correlation coefficient(r) [18].

RESULTS AND DISCUSSION
As shown in (Table 1 and 2), and based on t-test analysis of significance, there were a highly significant difference (p<0.01) in the mean percentage of c-Myc and p53 proteins expression in tissue of colorectal carcinoma (CRC) and colorectal normal tissue (CRN). In addition (Table 3) show the immunoeexpression of c-Myc and p53 protein in patients CRC.
In patients and control, the current study found a significant correlation \((P<0.05)\) between the mean percentage of c-Myc and p53 proteins (Table 4). The expression of c-Myc and p53 were heterogeneous dark brown nuclear staining in the tissue, as shown in Figure 1.

Table 1: Comparison of mean percentage of c-Myc protein among studied group

<table>
<thead>
<tr>
<th>Studied groups</th>
<th>N</th>
<th>Mean± Std. Error</th>
<th>Comparison of significant</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>P-value</td>
</tr>
<tr>
<td>Controls(CRN)</td>
<td>40</td>
<td>0.96± 0.04</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patients(CRC)</td>
<td>40</td>
<td>9.3± 1.70</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>80</td>
<td></td>
<td></td>
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</tbody>
</table>

Table 2: Comparison of mean percentage of p53 protein among studied group.

<table>
<thead>
<tr>
<th>Studied groups</th>
<th>N</th>
<th>Mean± Std. Error</th>
<th>Comparison of significant</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>P-value</td>
</tr>
<tr>
<td>Controls(CRN)</td>
<td>40</td>
<td>2.5± 0.3</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patients(CRC)</td>
<td>40</td>
<td>17.3± 0.6</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>80</td>
<td></td>
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</table>

Table 3: The immunoexpression of c-Myc and p53 protein in patients with colorectal cancer (CRC).

<table>
<thead>
<tr>
<th>Markers</th>
<th>Marker expression in patients ((N=40))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative N(%)</td>
</tr>
<tr>
<td>c-Myc</td>
<td>8 (20%)</td>
</tr>
<tr>
<td>P53</td>
<td>15 (37.5%)</td>
</tr>
</tbody>
</table>

Table 4: Pearson correlation \((r)\) between c-Myc and p53 in studied groups.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Studied groups</th>
<th>Correlation Coefficient (r) =</th>
<th>(P) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>c-Myc and p53</td>
<td>Controls(CRN)</td>
<td>0.257</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td></td>
<td>Patients(CRC)</td>
<td>0.155</td>
<td>&lt; 0.05</td>
</tr>
</tbody>
</table>

\(P<0.05\) = a significant difference
Figure -1: Immunohistochemical staining(IHC) of c-Myc and p53 proteins in tissue of colorectal carcinoma(CRC). Staining by DAB chromogen (dark brown) counterstained with nuclear fast red . (A) positive c-Myc immunostaining (X400). (B) positive p53 immunostaining.(X400).
The present study has shown increased expression of c-Myc and p53 in patients with colorectal carcinoma compared with healthy individuals. This result is consistent with previous reports that the overexpression of c-Myc, c-neu, PCNA, and p53 may occur in colorectal carcinoma (CRC) that are likely to metastasize. The expression of c-Myc has been detected in a broad range of human cancers to metastasize to the liver [18]. Deregulated expression of c-Myc is detected in many tumor cell types and it has been proposed that increased c-Myc expression is instrumental in the initiation of the neoplastic phenotype in many, if not most, human tumors. However, the mechanisms that normally regulate c-Myc expression, the defects that deregulate it in tumors, and how deregulated c-Myc expression contributes to tumorigenesis have not been fully elucidated [20]. Another study demonstrated that c-Myc overexpression may be sufficient to induce S-phase entry in a growth-arrested human cancer cell line. The transcriptional repression of p21 expression by c-Myc preceding entry into S phase. Thus the constitutive p21 overexpression inhibits deregulation of DNA synthesis by c-Myc, which suggests that the inhibition of p21 expression by c-Myc may contribute to its cell cycle promoting effect [21].

It is worth pointing out that p53 nuclear overexpression detected by immunohistochemical has been found to be a marker of worse prognosis in many previously published analyses of CRC datasets [22,23,24].

It is well known that tumor suppressors are formally defined by a loss of function involved in blocking tumor progression. Consistent with this definition, naturally occurring mutants of p53 are generally defective in sequence-specific DNA binding and consequently do not induce the appropriate target genes, cause cell cycle arrest, or mediate cell death [25]. However, in contrast to a classical tumor suppressor, mutation of the p53 gene leads not only to a loss of function but also to a gain of function that promotes the tumorigenicity of various p53-null cell types. Overexpression of mutant p53 in pre-B cells [26,27,28] fibroblasts [29], and osteosarcomas [30] dramatically enhances the tumorigenicity of these cells independent of a transdominant negative mechanism. In addition, stable expression of naturally occurring mutant p53 alleles in human T-cell acute lymphoblastic leukemia cells increases tissue invasiveness and enhances tumor formation [31].

Based on immunohistochemistry study, c-Myc was positively expressed in 80% (32/40) of group of colorectal cancer. This observation was consistent with another study that showed higher expression of c-Myc in colorectal carcinoma (68% in colon carcinoma and 55% in rectal carcinoma) by using immunohistochemistry [32,33]. On the other hand, p53 was positively expressed in 62% (25/40). Previous study
showed that from 244 colorectal tumors (55%) over-expressed p53 [34]. This might indicate the most important role of c-Myc and p53 in carcinogenesis of colorectal tumors.

The results of Mark et al demonstrate that tumor-derived missense mutants of p53 can regulate expression of the c-Myc gene. The well-established role of c-Myc [35] as a proto-oncogene capable of promoting cell cycle progression and tumorigenesis makes this gene an attractive target for p53 gain of function mutants, thus the efficient activation of the c-Myc promoter by mutant p53 occurs by a mechanism that is distinct from wild-type p53 transactivation [36].

In keeping with previous notions, our results showed that a significant correlation between c-Myc and p53. This might indicated that increasing expression of c-Myc with increasing expression of p53 that might reflect a pathological role of these two marker in colorectal tumor.

Conclusion: Our results confirms a significant association between colorectal cancer and increased expression of c-Myc and P53. In addition the evaluation of p53 overexpression, using a standardized immunohistochemical (IHC) procedure, could be a clinically useful marker for the identification of colorectal cancer patients.

REFERENCE


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