c-Myc oncoprotein expression and prognosis in patients with carcinoma of the cervix: An immunohistochemical study

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Summary

Objective: Evaluation of the prognostic significance of c-Myc expression in carcinoma of the cervix.

Materials and methods: 132 patients with carcinoma of the cervix presenting at the Cancer Institute in Chennai from 1996-1999 were included in the study. All the cases were treated with a radical course of radiotherapy and had a median follow-up of 36 months. Paraffin blocks obtained from the punch biopsies from the tumours were used for immunohistochemistry.

Results: c-Myc nuclear immunoreactivity was observed in 44/132 (33.3%) of tumours. Patients with tumours in Stages II B and III B accounted for 112/132 (84.8%) of the cases. Patients with tumours expressing c-Myc nuclear immunoreactivity were found to have more advanced disease; less likely to achieve complete remission (54.5% vs. 78.4%; $p < 0.005$) and lower disease-free status (52.3% versus 73.8%; $p < 0.014$). The poor prognostic feature of c-Myc nuclear immunoreactivity was seen in both stage II B (52.9% vs. 76.3%) and stage III B (38.1% vs. 69.4%; $p < 0.025$).

Conclusions: c-Myc nuclear immunoreactivity is a predictor of poorer response to radiotherapy and poorer disease free status in stage II B and III B carcinoma of the cervix.

Key words: c-Myc expression; Carcinoma cervix; Prognosis.

Introduction

Cervical cancer is the leading cause of cancer-related deaths in women in many developing countries including India. It is the most common cancer affecting South Indian women. The crude incidence rate is 26.1/100,000 according to the Madras Metropolitan Tumour Registry. Chennai (Madras) is one of the highest prevalence areas of cervical cancer in India [1]. More than 80% of our cases present with the disease in advanced stages of II B and III B and more than 90% belong to high grade. It is therefore important to establish new predictive markers which would be useful in determining a reliable prognosis in the locally advanced stages of the disease so as to minimize failure rates. A method which can be applied to the tissue sections to identify these markers should be emphasized rather than one which would involve sophisticated and specialized technology. We have evaluated c-Myc oncoprotein expression as one such marker by immunohistochemistry.

The c-Myc protein is a 62 kD phosphoprotein that complexes with another cellular protein Max to form a DNA binding complex that interacts with a consensus nucleotide suquence CACGTG. This protein plays a role in regulation of the cell cycle particularly at the onset of proliferation, in transformation and in differentiation [2]. CACGTG motif was previously identified as the binding site for transcription factors containing basic region and helix-loop-helix motifs. c-Myc oncoprotein contains these motifs enforcing its role as a transcription factor [3]. The mechanism by which these processes are operational involves elevated expression of the c-Myc protein probably leading to enhanced transcription of various genes involved in proliferation and cell growth. c-Myc protein is biologically stable with a half-life of 15-20 min [4]. c-Myc oncoprotein is continuously expressed in cell cycles and the levels of this protein vacillate in response to the agents stimulating or repressing proliferation. In contrast to c-fos and c-jun, c-Myc levels are maintained at constant levels throughout all phases of the cell cycle in continuously proliferating cells. Cells constitutively expressing c-Myc have reduced growth factor requirements, increased growth rate and can circumvent cell cycle arrest [5]. Proliferation and differentation represent two mutually exclusive events and c-Myc probably plays a pivotal role in this decision making process. c-Myc expression is generally low and undetectable in differentiated adult tissues, consistent with the notion that c-Myc expression correlates with cell proliferation. Alterations of c-Myc have been reported in a variety of cancers, like colon cancer, lung cancer and glioblastoma. Protein stabilization has also been reported in human glioma cell lines [6]. The common phenomena found in all these tumours was the inability to downregulate the expression of c-Myc in response to differentiating agents and eventually obtaining increased potential to undergo a cell division cycle, leading to additional somatic mutations and tumour burden.

Here we have studied 132 cases with invasive squamous cell carcinoma of the cervix for c-Myc immunoreactivity and analysed their prognostic significance.

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Materials and Methods

Patient materials

Paraffin blocks were obtained from 132 cases with invasive squamous cell carcinoma presenting at the Gynecologic Oncology Clinic of the Cancer Institute between 1996 and 1999. All these cases were treated with a radical course of radiotherapy and had a minimum follow-up of 16 months, median follow-up of 36 months and a maximum follow-up of 54 months.

Immunohistochemistry

Sections from formalin-fixed, paraffin-embedded blocks were immunostained using an avidin-biotin peroxidase complex (ABC) method. Briefly, the sections were dewaxed in xylene and dehydrated in absolute alcohol. The endogenous peroxidase activity was quenched using 30% hydrogen peroxide and non specific binding was blocked using 2% bovine serum albumin in phosphate buffered saline. The sections were incubated in anti c-Myc mouse monoclonal (9E10, Santacruz, 10 µg/ml) overnight at room temperature. Following sequential incubations with biotinylated secondary antibody and avidin-biotin peroxidase complex, the peroxidase reaction was developed using diaminobenzidine as a chromogen. All our series had a normal colon tissue section as positive control [7] and for negative control the primary antibody was omitted. All the controls gave satisfactory results. The slides were independently scored by TR and NV and where the scoring was not concordant, a joint review was done. The tumours were scored based on the pattern of immunoreactivity: negative, cytoplasmic only, and nuclear reactivity. All tumours with nuclear immunoreactivity showed cytoplasmic staining as well. Tumours were considered to be positive only if they had nuclear immunoreactivity. Among the cases positive for c-Myc expression, the levels of expression were coded as follows: less than 10% of tumour nuclei expressing c-Myc, 10-25% of tumour nuclei expressing c-Myc, 25-50% of tumour nuclei expressing c-Myc and 50-75% of tumour nuclei expressing c-Myc.

Statistical Analysis

The product limit method of Kaplan and Meier was used to analyse the survival periods. The Chi square test was used to assess the statistical significance of the correlation of c-Myc immunoreactivity with clinical parameters.

Results

The pattern of expression of c-Myc oncoprotein analysed by immunohistochemistry in 132 cases was as follows. Both nuclear as well as cytoplasmic expression were seen in 44/132 (33.3%) cases. Cytoplasmic expression alone was seen in 42/132 (31.8%) cases. No nuclear or cytoplasmic reactivity was observed in 46/132 (34%) cases; 44/132 (33.3%) were positive for c-Myc nuclear immunoreactivity and 88/132 (66.6%) were negative for nuclear immunoreactivity. Comparsion of nuclear immunoreactivity with clinical parameters like age, clinical stage, histologic grade is shown in Table 1. Expression of c-Myc nuclear immunoreactivity was found to rise with increasing stage. The increase in the number of tumour nuclei expressing c-Myc was inversely associated with the disease-free status (Table 2). Patients with tumours having c-Myc nuclear immunoreactivity failed to achieve complete remission and had a higher failure rate (20/44

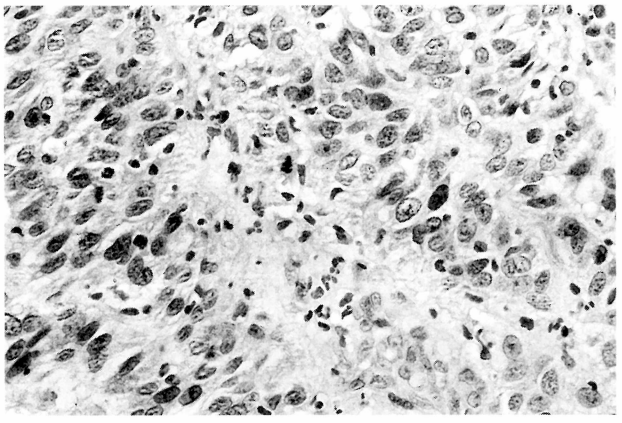

Fig.

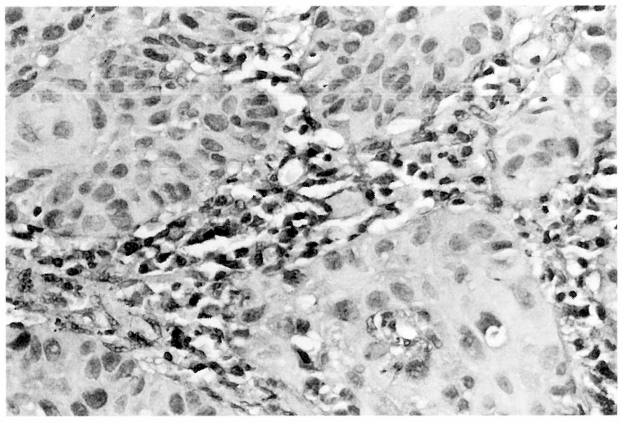

Fig.

Figure 1. — Cervical tumour section stained for c-Myc oncoprotein showing positive immunoreactivity in the nuclei.
Figure 2. — Cervical tumour section stained for c-Myc oncoprotein showing no nuclear or cytoplasmic immunoreactivity.

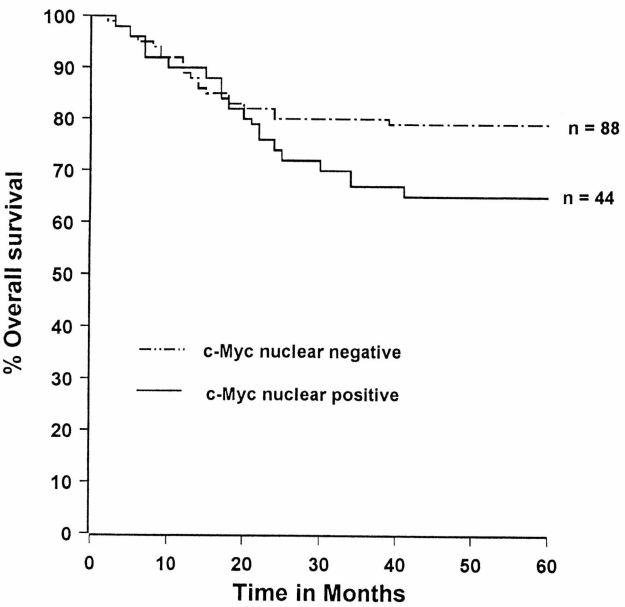

Figure 3. — c-Myc expression vs overall survival.

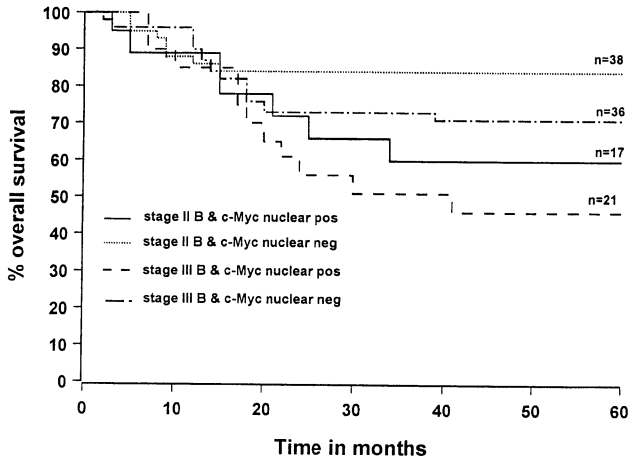

Figure 4. — c-Myc expression in stage II B & stage III B vs overall survival.

Table 1. — *Clinical parameters versus c-Myc expression.*

Clinical parameters	c-Myc nuclear - VE	c-Myc nuclear + VE
Age (years)		
25-35	15 (62.5)	9 (37.5)
36-45	36 (67.9)	17 (32)
46-55	24 (68.5)	11 (31.4)
56-65	12 (66.6)	6 (33.3)
> 66	1 (50)	1 (50)
Stage		
Stage I B (n=7)	5 (71.4)	2 (28.5)
Stage II A (n=12)	9 (75)	3 (25)
Stage II B (n=55)	38 (69)	17 (30.9)
Stage III B (n=57)	36 (63.1)	21 (36.8)
Stage IV A (n=1)	0	1 (100)
*Grade**		
Low Grade (I & II)	6 (60)	4 (40)
High Grade (III & IV)	71 (67.6)	34 (32.3)

* Grade was not evaluable in 17 cases. The numbers within brackets denote percentages.

Table 2. — *c-Myc pattern of expression versus outcome*

Description (p < 0.005)		Disease free	Failure
Nuclear positive	(n=44)	23 (52.3)	21 (47.7)
Less than 10%	(n=16)	12 (75)	4 (25)
10-25%	(n=15)	8 (53.3)	7 (46.6)
25-50%	(n=10)	2 (20)	8 (80)
50-75%	(n=3)	1 (33.3)	2 (66.6)
Nuclear negative	(n=88)	65 (73.8)	23 (26.1)

The numbers within brackets denote percentages.

Table 3. — *c-Myc nuclear expression versus remission and outcome status*

Response (p < 0.005)	CR	PR
c-Myc nuclear - VE	69 (78.4)	19 (21.5)
c-Myc nuclear + VE	24 (54.5)	20 (45.5)
Outcome (p < 0.014)	NED	Failure
c-Myc nuclear - VE	65 (73.8)	23 (26.1)
c-Myc nuclear + VE	23 (52.3)	21 (47.7)

The numbers within brackets denote percentages; NED = no evidence of disease; CR = complete remission; PR = partial remission.

[45.5%] and 21/44 [47.7%]), respectively, than patients with tumours lacking c-Myc nuclear immunoreactivity (19/88 [21.5%] and 23/88 [26.1%]), respectively (Table 3). Of the 132 cases, 112 cases belonged to stage II B and stage III B (84.8%). In stage II B, 9/38 (23.7%) were found to fail treatment among cases showing no nuclear immunoreactivity compared to 8/17 (47.1%) cases showing c-Myc nuclear immunoreactivity. In stage III B, 11/36 (30.6%) were found to fail treatment among cases showing no nuclear immunoreactivity for c-Myc compared to 13/21 (61.9%) among cases showing positive c-Myc nuclear immunoreactivity [p < 0.025] (Table 4). Survival periods calculated by the Kaplan Meier product limit method showed a better overall survival among cases showing no c-Myc nuclear immunoreactivity compared to cases expressing c-Myc in the nucleus (Figure 3). The survival periods compared between stages II B and III B versus nuclear immunoreactivity also showed the patients with tumours exhibiting c-Myc nuclear immunoreactivity as having a poorer overall survival compared to patients with tumours which were negative for nuclear immunoareactivity (Figure 4).

Discussion

Cervix cancer is the most common cancer seen in South Indian women. More than 80% of cases present in stage II B and III B. Despite adequate management, more than 50% fail treatment. Clinical and pathological prognostic markers like the stage and histologic grade do not appear to be contributory as more than 80% are in stage II B and III B and nearly 90% are high grade squamous cell carcinomas. Identification of a molecular marker which can be studied by a simple technique, not involving any other clinical material other than the routinely obtained paraffin block from the patient could be immensely useful in predicting the course of the disease, parti-

Table 4. — *Stage II B & III B remission and outcome status versus c-Myc nuclear expression*

	STAGE II B				STAGE III B			
	CR	PR	Disease free	Failure	CR	PR	Disease free	Failure
c-Myc nuc – VE	31 (81.6)	7 (18.4)	29 (76.3)	9 (23.7)	26 (72.2)	10 (27.7)	25 (69.4)	11 (30.6)
c-Myc nuc + VE	9 (52.9)	8 (47.1)	9 (52.9)	8 (47.1)	9 (42.8)	12 (57.1)	8 (38.1)	13 (61.9)

CR = complete remission; PR = partial remission. The numbers within brackets denote percentages; [p > 0.025]

cularly when the presentation is in higher stage and grade. This is the first study from the Indian population, where we report c-Myc nuclear immunoreactivity to be an unfavourable prognostic indicator and a predictor of poorer response to radiotherapy in stage II B and stage III B. Our study has also shown that an increase in percentage of tumour cells showing nuclear immunoreactivity was significantly associated with failure of treatment. c-Myc overexpression and its relevance in cervical cancer as a predictive and prognostic marker have been reported earlier for a fewer number of cases [8]. c-Myc was reported to be an independent prognostic variable for early stage invasive cervical cancers in a study of 94 cases, where c-Myc expression was reported to be predictive of early recurrence and failure [9-11]. Our results showed the increase in the percentage of tumour nuclei expressing c-Myc to be inversely related to disease free status and an indicator of failure. The number of cases positive for c-Myc expression was also found to increase with the increase in the stage in our series thus suggestive of a role for c-Myc in the progression of the disease. This was confirmed earlier by Nair *et al.* [12]. They studied the cellular alterations of myc genes during evolution from the preneoplastic to neoplastic lesions by describing its differential expression but their role as a prognostic variable was not reported. Prognostic significance of c-Myc was also studied by northern and slot blot techniques in 30 cases. Patients with 10 times higher c-Myc mRNA compared to normals relapsed and died within two years of treatment suggesting overexpression of this oncoprotein was associated with poor survival [13]. Overexpression of this oncoprotein has also been attributed to gene amplification and gene rearrangements which were found in 90% of the cases [14]. Blotting techniques are difficult for routine studies unlike immunohistochemistry which is a simple yet semiquantitative technique where contamination with normal cells is not a setback.

c-Myc levels from CINs to invasive tumours were analysed in 31 cases and better DFS was found among the c-Myc negative cases. The 16 invasive cancers with positive c-Myc immunoexpression were found to develop extra pelvic metastatic disease [15]. A few studies have evaluated associations of c-Myc with other molecules. Expression of c-Myc, and its association with EGFR, c-erbB2 and its association with the clinical prognostic factors was assessed in seven cervical adenocarcinomas and were reported to have no prognostic significance [16]. Associations of c-Myc expression, cyclin E and p27kip1 with progression and prognosis in cervical neoplasms were studied in 69 cervical neoplasms and although c-Myc expression was correlated to cell proliferation in precancerous lesions it was not correlated to the overall survival [17].

Conclusion

Positive nuclear immunoreactivity for c-Myc oncoprotein is an indicator of poorer response and survival in stage II B and stage III B carcinoma of the cervix. The increase in the number of tumour nuclei expressing this oncoprotein is directly related to poorer response.

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