

Biomarkers in Carcinoma of the Cervix: Emphasis on Tissue-related Factors and Their Potential Prognostic Factors

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Abstract

Despite the introduction of the PAP smear screening technique, cervical carcinoma continues to be a significant disease worldwide in terms of prevalence, morbidity and mortality.

This paper reviews the prognostic value of biomarkers from some oncogenes, including c-myc, ras and c-erb B-2, the cellular proliferation markers PCNA and Ki-67, and other more recently described biomarkers such as nm23-H1, MN protein and metalloproteinase. Emphasis is given at a practical level to markers which can preferentially be applied to tissue sections rather than involving other modalities of investigation which may require specialised equipment and technology. No single marker of those previously listed was found to have outstanding prognostic significance. Although some have shown promise in initial studies subsequent investigations have not provided corroborating evidence, or, in some situations, have also led to conflicting results. Difficulties inherent in establishing the prognostic value of individual markers also include the multifactorial complexity of cervical carcinogenesis itself. The future awaits a greater amount of data to be accrued across all stages of disease, with improved standardisation of results.

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Introduction

Accurate staging is of utmost importance in determining the prognosis of carcinoma of the cervix. Demographic features such as race and socio-economic status have been demonstrated as not having significant influence.¹ The prognostic significance of younger age is controversial. Traditionally, histologic parameters are used to prognosticate and modify management options. Of these, tumour size or volume, depth of tumour invasion, lymph node status and vascular channel or surgical margin involvement are well established factors.² The histologic type of tumour plays a minor role, with the exception of a few uncommon specific tumour types associated with highly aggressive or indolent behaviour. The significance of the degree of tumour angiogenesis remains to be elucidated, some studies showing that microvessel density carries independent prognostic significance,^{3,4} while others find that its significance is correlated with tumour intravascular space invasion.⁵

As with other neoplasias, biomarkers are being increasingly called upon to provide possible prognostic information especially in those elusive cases with exceptional or discordant behaviour, in an attempt to better

understand the disease profile, as well as the potential application of individualised therapy. This is of particular relevance in stages of the disease for which there are several therapeutic options, such as stage Ib carcinoma of the cervix.

In this review, biomarkers from oncogene products, cell proliferation and proteinases are examined for their prognostic significance.

Oncogenes

C-myc

The myc oncogenes function as nuclear DNA transcription factors associated with cell cycle progression, immortalisation and differentiation. Adding to its functional complexity, c-myc also has a capacity for transcriptional regression and an additional role in apoptosis.⁶

C-myc is expressed in both cervical intraepithelial neoplasia (CIN) and invasive carcinoma, with varying levels of expression according to the extent of the disease. Devictor et al⁷ demonstrated c-myc immunohistochemical (IHC) positivity in 19% of cases of CIN3 and 70% of microinvasive squamous cell carcinoma (SCC). In frankly invasive tumour, the percentage of cases showing c-myc amplification by molecular techniques is

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generally higher compared with IHC methods, but the range is wide (32% to 90%)^{8,9} Overall, approximately 50% of invasive tumours show expression of c-myc oncogene.^{10,11}

In early cervical cancer, c-myc over-expression was found in 12.5% and 33% of the tumours. This has been found to be associated with worse prognosis.^{12,13} In an advanced stage series, c-myc was immunohistochemically expressed in 45.1% of the tumours, but may not have prognostic import.¹⁴

Ras

The Ras family of oncogenes code for transducing proteins. One such product is the membrane-associated protein p21 which possesses guanine triphosphatase (GTPase) activity. The putative role of mutant ras is to convert human papillomavirus (HPV)-immortalised keratinocytes to the tumorigenic state.¹⁵

Compared with normal tissue and lower grades of dysplasia, Ras over-expression is found more commonly in CIN3 and invasive carcinoma, with IHC positivity ranging from 39% to 100%.^{16,17}

It has been reported that the presence of the oncogene product p21 in early stage cervical tumours correlates with the presence of lymph node metastases, and hence poor prognosis.¹⁸ Sagae et al¹⁶ suggested p21 positive large cell type squamous cell carcinoma has a poorer prognosis, and small cell type a better prognosis in comparison with the p21 negative cases. On the other hand, Symonds et al¹⁴ found no association between oncogene expression, local recurrence or survival in their series of advanced stage cases.

C-erb B-2

The C-erb B-2 proto-oncogene codes for a transmembrane tyrosine kinase 185 kD oncoprotein which is related to the epidermal growth factor receptor (EGFR). Although it has been suggested that higher levels of C-erb B-2 expression are seen at later stages of carcinoma,¹⁹ analysis of the reported positive cases does not bear this to be true.

A relatively high rate of IHC positivity (26%) is seen in late stage disease.²⁰ But both lower (12.1%) and higher (38.7%) positivity rates have been described in early stage cases.^{21,22} These series, however, utilised different monoclonal antibodies and were composed of different tumour types, with lower rates reported in tumours of purely squamous origin.

The tendency for studies reporting a relatively higher rate of C-erb B-2 positivity to find a significantly poorer survival,^{20,22,23} whilst series with a lower incidence of positivity reported no survival significance,²¹ indicates clinical limitations of the test depending on laboratory methodologies.

Epidermal Growth Factor Receptor

The EGFR is an oncogene-encoded 170 kD membrane-bound glycoprotein, of importance in the regulation of normal and neoplastic cellular proliferation. Activation of the receptor's intracellular tyrosine kinase domain functions to transmit intracellular signals leading to regulation of cell growth.

EGFR expression has been examined in relatively few cases of normal and dysplastic epithelium with contradictory results. Dellas et al²⁴ finding of abnormal expression in CIN compared with normal squamous epithelium contrasts with Kim et al²⁵ and their lack of discrimination between EGFR values in normal and dysplastic epithelium. Different techniques were, however employed by these authors; the former utilising IHC and the latter utilising enzyme-linked immunosorbent assay (ELISA).

The situation is no clearer in invasive carcinoma, with reports demonstrating lack of significance of tumour EGFR expression for survival,²⁶ countered by poor survival in positive cases.^{21,27} These were not, however, equivalent studies in terms of tumour stage and histologic types examined.

p53

The normal or wild type tumour suppressor gene p53, named for its molecular weight of 53 kD, normally plays a role in regulation of the cell cycle and cell loss through apoptosis, and is not usually detectable immunohistochemically. By contrast, the mutated form of p53 is rendered detectable as it has a longer half-life than the wild type, and accumulates within the nucleus following stabilisation.²⁸ Non-detection of p53 despite the presence of mutation is possible when the mutation results in non-production of the protein or the threshold for detection is too low.

In carcinoma of the cervix there are two types of alteration of p53 expression; inactivation of wild type p53 by binding with the protein product of E6 gene of HPV 16, and less commonly spontaneous mutation of p53 in HPV negative tumours.

Thus in HPV positive cervical carcinoma there should be an inverse relationship with p53 secondary to p53 degradation as the result of binding with the E6 HPV oncoprotein. Findings do not, however, universally support this mutually exclusive view and exceptions abound. A recent paper emphasises the lack of correlation between the immunostaining for p53 and the HPV 16/18 tumour status.²⁹ Mittal et al²⁸ found the simultaneous presence of p53 and HPV in overall 17% to 50% of lesions, including condylomata and neoplasias. The authors do, however, admit to their inability to determine if the p53 detected was of wild or mutant type.

The situation is further complicated by some HPV positive carcinomas which may additionally demon-

strates spontaneous p53 mutations,³⁰ and some HPV negative carcinomas which lack spontaneous p53 mutations.³¹

In summary, p53 is of seminal relevance for its role in cellular oncogenesis and relationship with HPV, but has not been demonstrated to have any prognostic significance.

nm23-HI

The nm23-HI gene is a metastasis suppressor gene, the product of which is identical to human nucleotide diphosphate (NDP) kinase A. The function of this protein remains unclear; it has been suggested that it modulates intracellular signal transduction by phosphorylation of GTP binding proteins, or plays a role in cell attachment and detachment to the extracellular matrix.³²

A possible intriguing association of nm23-HI expression with a high incidence of lymph node involvement and poor prognosis has been described in the adenocarcinoma subtype of tumours only.³² However, when a larger number of cases were examined by Kristensen et al³³ by a similar IHC method for detection of nm23/NDP kinase protein, more frequent staining was observed in cervical tumours with a squamous component than in adenocarcinoma. This study also refuted the claim of an association with lymph node metastasis.

Clearly, at this early stage of investigation, no direct relationship of the nm23-HI metastasis suppressor gene product with cervical tumour type and behaviour is demonstrable, less promising than its role for tumours originating in other sites.

Cell Proliferation Markers

Cell kinetics analysis of tumours can be used to provide potential prognostic information. Early cell kinetic experiments measured incorporation of tritiated thymidine into DNA during S phase. The pre-treatment proliferative activity of SCC of the cervix, as assessed by an immunofluorescent method of S phase fraction determination by anti-thymidine antibodies was found to correlate significantly with disease activity.³⁴

Complete regression was observed in the majority of rapidly proliferative tumours, compared with 41% of slowly proliferative tumours, all of which were treated with radiotherapy. Thus, in addition to prognostic information, this study also attested to the beneficial effects of high tumour proliferation rate to success of irradiation therapy.

Measurement of cell proliferation can also be made on the levels of cell cycle-associated structural or functional moieties.³⁵ Tissue sections can be analysed for these cell cycle-related antigens using antibodies.

An important cell protein is cyclin/proliferating cell nuclear antigen (PCNA) which accumulates progressively through interphase and is a component of a DNA polymerase, essential for completion of the cell cycle.

Cyclins are a family of proteins implicated in the induction and control of mitosis, activating cell division cycle (cdc) kinases. The antibody known as cyclin or PCNA is expressed during the G1 and early S phases of the proliferation cycle, but its utility may be diminished by its demonstration in post-mitotic cells.³⁶ Mittal et al³⁷ found that the percentage of basal cervical epithelial cells expressing PCNA increased progressively from atrophic, normal, and condylomatous through to dysplastic squamous epithelium. The level of proliferating PCNA positive cells within epithelium has also been found to progressively increase from normal cervix, and through CIN1 to CIN3.³⁸ Its prognostic significance is, however, uncertain.^{39,40}

One of the earliest, and possibly best described antibodies which recognises proliferating cells is Ki-67, which identifies a nuclear antigen associated with the cell cycle, being expressed in all phases of cell replication except Go. A major practical limitation however, has been the requirement for fresh tissue, as the antigen is destroyed by formalin fixation.

As with PCNA, the upper level of Ki-67 positive cells in squamous mucosa increases in CIN in contrast with non-neoplastic cervix. Ki-67 immunostaining is also significantly greater in invasive SCC, compared with CIN.^{7,41}

A relationship between increased quantitative expression of Ki-67 and tumour size in SCC has been corroborated in cases of micro-invasive,⁷ and early stage disease.^{41,42}

Proteinases

Cathepsin D

The relatively scant IHC studies in cervical carcinoma of this acid proteinase have produced conflicting results. Kristensen et al²¹ found positive staining in SCC (47% of cases) to correlate with lymph node metastases and lower relapse free survival, with the immunohistochemical result achieving independent prognostic significance. This is in disagreement with the finding of Mitchell et al⁴³ for invasive carcinoma despite a similar percentage of positive immunostaining. As cathepsin acts to degrade extra-cellular matrix and activates other proteinases involved in early invasion, dysplasia and microinvasive stage 1 malignancies may be more fruitful areas for investigation.

Lack of high levels of expression in established invasive malignancy may highlight the supervening importance of other co-factors required for the development of metastases, or be related to levels at which the proteinase is detectable in tissue sections.

MN Protein

Expression of this protein has only recently been detected in cervical neoplasia, and may correlate with loss

of a tumor suppressor gene on chromosome 11, possibly at an early stage of oncogenesis.⁴⁴

The membrane-associated MN antigen (Ag) has homology with carbonic anhydrase. Although MN protein expression in cervix carcinoma is ubiquitous, there is quantitative variation, with low expression found to correlate with poor differentiation, the adenosquamous histologic subtype, deep stromal invasion, regional lymph node metastases and HPV negativity.⁴⁵

The numbers of recurrent carcinoma cases studied to date is also too small from which to draw conclusions, and the role of this biomarker protein as a prognostic marker in cervical carcinoma is therefore yet to be determined. A possible hindrance to further studies at this stage with this novel biomarker is also lack of a commercially available antibody.

Metalloproteinase

Metalloproteinase (MP) may help to degrade basement membrane type IV collagen, assisting the early phases of tumour invasion, and thereby potential for metastasis. Garzetti et al⁴⁶ have demonstrated a significant increase in IHC positive in microinvasive squamous cell carcinoma compared with CIN lesions. Increased staining positivity in CIN also related directly to the severity of cellular atypia.

A significant relationship has been claimed between the tumour MP index and presence of nodal metastasis, as well as the number of positive nodes and recurrence risk.⁴⁷ Overall early results seem promising but will require additional confirmatory evidence.

Conclusion

In carcinoma of the cervix, there is an over-expression of the c-myc, ras, c-erbB-2 and EGFR oncogenes, but these are not of consistent value in determining prognosis at this stage. Neither have tumour HPV status and presence of the tumour suppressor gene p53 been demonstrated to have any prognostic significance.

Quantification of the cell proliferation markers may be of benefit in cases of dysplasia of the cervix in distinguishing CIN from benign simulators. Further studies await determination of their role, as well as that of other biomarkers Cathepsin D, nm23-H1, MN protein and metalloproteinase, as prognostic indicators.

At the early stage of investigation of some of these markers, comparison between studies is hampered by unequivalent database groups, lack of uniform end points and differences in methodologies used in the evaluation of each parameter. As to be expected, no one biomarker appears to be of outstanding merit. An integration of results may eventually yield information of improved predictive value than the standard histopathologic parameters.

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