Review Article

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ROLE OF TUMOR MARKERS IN CERVICAL CARCINOGENESIS

J. S. Misra, A. N. Srivastava, Shivani Singh

Department of Pathology

Era's Lucknow Medical College & Hospital, Era University, Sarfarazganj Lucknow, U.P., India-226003

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Address for correspondence

Dr. J.S. Misra
Department of Pathology
Era's Lucknow Medical College & Hospital, Era University, Lucknow-226003.
Email: jata_misra@yahoo.com
Contact no: +91-9005917960

ABSTRACT

Various types of tumor markers are currently being investigated to ascertain their capability in discriminating pre-cancerous lesions of cervix who have tendency for progression. The adequate treatment of such cases will check any chances of occurrence of carcinoma cervix in the population. The micro- RNAs are sensitive tumor markers but their high cost and sophisticated technique make them not feasible to be introduced in any cervical cancer screening program under Indian setup. Other tumor markers like claudins, p16, Ki67 etc are also very expensive. AgNOR pleomorphic counts and micronuclei counts are cheaper, the farmer being more reliable can be introduced in cytological screening program to identify high risk cases and can easily replace costly Human papilloma virus (HPV)- DNA testing.

KEYWORDS: Micro-RNAs, Stem cell markers, Protein markers, High risk SIL cases, AgNOR. Pleomorphic counts.

INTRODUCTION

Though cervical cytology has been widely used in the diagnosis of carcinoma cervix in its pre-invasive phase, but the future biological behavior of premalignant lesions is not known. This has been made possible by the application of different tumor markers which are either proliferative or suppressive and their increased activity in the premalignant conditions of cervix and their association with progression or regression of the lesions has been ascertained to find out their potential to discriminate high risk cases which could progress to higher grade and eventually to carcinoma cervix. The researches in the last three decades have led to the invention of different tumor markers for evaluating their diagnostic or prognostic potential in cervical carcinogenesis.

It is well established fact that 80% of SIL cases regress spontaneously or after treatment and only 20% of them either persist or progress to higher grade. In all probability, these 20% cases might be high risk HPV-positive. If these 20% of SIL cases can be picked up from the pool of SIL patients detected during screening, it will not need follow up of all detected SIL cases, therefore enhancing the efficiency of cervical cytology. Applications of different tumor markers have made it possible to detect these 20% SIL cases, the timely treatment of which would check any malignant transformation of cervix.

Many tumor markers have been tried in the last three decades to study their expression with increasing severity of pathological lesions of cervix. These are categorized in following groups-

1) DIAGNOSTIC ANTIGEN SERUM MARKERS

These tumor markers are used in the clinical laboratories in diagnosing patients with carcinoma cervix. There are following four types of such tumor markers-

1. Abnormal levels of SAC- Ag are present in 64% of the patients with Squamous cell carcinoma (1).
2. Cancer antigen125 elevated levels are seen in the SIL cases. As reported by Ngan et al, the abnormal levels of CA-125 was detected in 18% of the squamous cell carcinoma (2). In squamous cell carcinoma, the abnormal level of CA-125 was detected in 18% of the patients.
3. Borras et al have found CA19-9 more sensitive marker in squamous cell carcinoma and able to detect when reported negative by CA-125. (3)
4. Cytokeratin 19 fragment 21-1 (CYFRA21-1) is useful marker than other cancer antigens for its specificity in helping the diagnosis of carcinoma cervix and its increased level is associated with tumor staging system.(4)

2) DNA METHYLATION MARKERS

In these markers, methyl group is added at 5' position of cytosine with the help of enzyme methyl transferase. These markers are basically involved in inhibition of gene expression and its expression is

increased only in pre-invasive lesions and frank carcinoma and hence could be employed for prognosis in cervical cancer screening. Some of the tumor markers formed as result of methylation of some particular gene are given below-

a) Hosnil, J et al (2007) have evaluated hypermethylation of following 14 genes for their involvement in cervical carcinogenesis.(5) These are – p16, hmLH1,Cox-2, pk1,RASSFIA ,TIMP-3, THBS-1,HLTF etc. The authors have found only COX-2 gene effective in the prognosis of pre-invasive phase of cervical cancer.

b) Widschwendter, A et al have studied aberrant methylation of gene- CDH-1 and CDH-3 in cancer cervix cases and have found it as useful marker in finding relaxed cases.(6)

c) Wentzensen, N et al have found three markers-DAPK-1, CADM-1 and RARB showing consistently elevated methylation in cervical cancer patients.(7) These methylation markers are not so effective to be employed in cancer screening program.

3) MICRO RNA MARKERS

Micro-RNAs are regulating by enzyme RNAase that controls gene expression at the posttranscriptional level. Certain micro- RNAs have been found located near the cancer susceptibility loci that correlate tumorgenesis. Epigenetic abnormality like disregulation of micro- RNA is common in the carcinoma cervix patients. A number of micro- RNAs have shown a consistent upregulation (Micro-RNA 29a) or downregulation (Micro-RNA 21) through different stages of cervical cancer.(8) The other Micro-RNAs like miR-349, miR-125 and mi-375, miR-109, miR20b, miR9, miR16 and miR106 have been found disregulated in cervical epithelial cells in relation to cancer progression.

Luo, M et al have found miRNA -497 downregulated in carcinoma cervix cases by transcription of IGR-IR protein expression.(9) Disregulation of other miRNAs have been found during malignant transformation of cervix-

miR299, miR222, miR185, miR146a, miR209, miR203, miR205, miR218, miR21, miR29a, miR200, miR25, miR485.

Certain Micro-RNAs show single nucleotide substitution in greater than 10% frequency and could be used in diagnosis of different types of cancer. Shi et al and Xiong et al have found variant of pre-miR -218 and pre-miR-27a is associated with the reduced risk of carcinoma cervix.(10)(11)

4) EMBRYONIC STEM CELL MARKER

The stem cell transcription factors in the cervical cancer has been focus of interest in answering this question. Zheng, P S has demonstrated that the stem cell related genes like OCT4, SOX2, BMI1 and LGR5 are increased in the process of development of cervical carcinogenesis while SOX 9, UTF1 and KLF4, on contrary, have been found as tumor suppressor.(12) Zing et al have found the SOX2 and OCT4 are activated in cervical carcinogenesis and squamous cell carcinoma and their expression levels are highly increased in cancer cells than in normal tissues.(13) The SOX2 expression was found to have a correlation between differentiation and patient status of survival in advance carcinoma cervix cases and hence have been considered as novel predictor for poor prognosis in cervical carcinoma patients.

5) TRANSCRIPTION FACTORS

a) Transcription factors are enzyme involved in the replication and DNA segregation. One such enzyme like Mini-chromosome maintenance protein-2 (MCM2) is overexpressed in the ascending grades of carcinoma cervix (Santin, et al, Murphy et al). (14) (15) An immunocytochemical assay – Pro Ex TM which has ability to detect HSIL through application of transcription enzyme like Topoisomerase IIA (TOP2A) and MCM2 in the cervical samples of carcinoma cervix (Malinowski et al). (16) Shroyer et al have also found this test useful in accurate diagnosis of cervical materials especially detecting CIN2+ lesions in LSIL diagnosed biopsies.(17) Tambouret et al have also found that ability to detect CIN 2+ with any level of cytological atypias was high with Pro Ex C. (18) Zheng et al have found the MCM2 expression level correlated with high risk HPV type and hence have opined utility of MCM2 as marker in cervical carcinogenesis.(19)

b) The Forkhead Box MI (FOXMI) is transcription enzyme is associated with regulation of expression of cell cycle gene in the cell cycle. Chan et al have found overexpression of this factor in carcinoma cervix than in normal samples indicating that FOXMI is associated with pre-cancerous and cancerous transformation of cervix.(20)

c) GINS complex subunit 2 (GINS2) is associated with DNA replication and is upregulated in many aggressive cancer types. Ouyang, F et al have found GINS2 unregulated in the carcinoma cervix cell lines than in normal samples. They have suggested that this can be a valuable biomarker in the prognosis of pre-cancerous lesions of cervix early and treatment. (21)

6) PROTEIN MARKERS

a) p53 is a protein commonly observed in the different pre-cancerous and cancerous stages of human cervix and has tumor suppressor quality. Sandhu, et al have observed 86.7% of p53 positivity in cervical cancer patients and only 10% in the normal cervical epithelium.(22) A CIN 3 case was also found p53 positive. Their study showed strong significant association of p53 positivity with cervical cancer. However, Deffar, K et al have found p53 positivity in only 24.6% of cervical cancer patients and have suggested that its non-suitability as prognostic marker in carcinoma cervix (23). Herbsleb, M et al have found p53 related to the cervical dysplasia but its increased level was seen in only CIN-3 and CIS cases.(24)

b) Bcl2 is encoded by Bcl2 gene in human and regulate cell death (apoptosis). Its overexpression is observed in the different stages of development of carcinoma cervix. It appears that changes in the Bcl2 expression occurs relatively early in the development of cervical cancer Aletra, et al.(25) Dimitrakakis et al have found Bcl2 expression increasing directly in relation to CIN grade and have noticed that increasing pattern of a Bcl2 protein pattern may be helpful in identifying high risk cases which need immediate follow up and treatment.(26)

Kamaraddi et al have found 75% positivity of Bcl2 in malignant lesions and 62.5% in premalignant cases. In CIN lesions, the positivity increased with the grade. They have found Bcl2 as capable of identifying high risk CIN cases which need further follow up.(27) Deffar et al have found moderate expression of Bcl2 in 10.4% of cervical cancer patients but they still thought that it might represent important indicator for cervical carcinoma.

d) MIB-1 (MOLECULAR IMMUNOLOGY BORSTEL) The proliferative index of this tumor marker has been used as substitute for diagnostic cervical cytology in ascertaining different stages of development of cervical cancer. Goel, M et al have found proliferative labeling index (PLI) increasing with increasing stages of SIL to cervical cancer with highest PLI seen in cancer patients.(28) They have suggested that this marker can be a used as a substitute for cytological diagnosis of cervical smears. Herbsleb et al have also found cervical dysplasia related to the MIB-1 and its increased level was seen in CIN2.

e) PCNA (PROLIFERATIVE CELL NUCLEAR ANTIGEN) Similar to MIB-1, proliferative activity of this marker has been found helpful in identifying ascending grades of SIL to carcinoma cervix. Goel, M et al have seen its proliferative index (PLI) increasing significantly with ascending grades of SIL and was highest in carcinoma cervix. Branca et al, have found PCNA expression increasing parallel to CIN grade with major upregulation occurring upon transition to CIN3. They have suggested that the increased PCNA expression was total indicator of CIN associated with high risk HPV infection.(29) Herbsleb et al have also found increased PCNA level in CIN1 cases only.

f) P16INK4a- This is a cell cycle regulated protein and is sensitive in SIL diagnosis and may be useful in finding SIL cases which can progress to higher grade. Janusicova et al have found raised P16 level in HSIL and frank cancer cases than HPV-16.(30) Izaaks et al have also observed a strong correlation between HPV infection and P16 positive samples (94.5%) and their study reinforced the value of P16 as surrogate marker in identifying women with progressive cervical disease.(31) Balan et al have also found that correlative analysis of P16 status and HPV expression could be helpful in assessing the progression of pre-invasive lesions of cervix.(32)

Zhao et al have suggested that P16 expression may be associated with HPV-16 status and their interaction can influence the progression of CIN to carcinoma cervix.(33) Reuschenbach et al have shown that the certain methylation of the viral genome is associated with nueoplastic alterations involving oncogenes E6 and E7. The p16 can diagnose this transforming stage of cervical carcinogenesis.(34) Shi et al have found association of P16 overexpression with high risk HPV infections in the development of cervical cancer and may be useful marker in the diagnosis and staging of CIN. (35)

Gattas et al have seen P16 protein expression increasing linearly from controlled cases to CIN peaking to squamous cell carcinoma and highlighting the importance of P16 analysis and HPV detection to find out CIN-1 cases who can progress to cervical neoplasia (36). Capobianco et al have also observed capability of P16 as a marker of pre-cancerous lesions of cervix and its expression increased with severity of cytological abnormalities. (37)

g) Ki-67 This protein being nuclear and nucleolar is indicator of increased proliferation of epithelial cells in the infected tissues and its enhanced activity may reflect infection of HPV. Kratig et al. have found a activity of Ki-67 increasing with ascending grade of SIL.(38)

Zhao et al have suggested from their findings that Ki-67 expression may be associated with HPV16 infection and their interaction can influence the progression of CIN. Turkcuoglu et al have found Ki67 expression increasing proportionally to the CIN grade and cervical carcinoma. However, this was not found

stastically significant and hence Ki67 expression could not be used in determining the aggressiveness of the lesions. (39) Similar opinion was also made by Eun et al who have found that the CIN grades were positively related to the Ki67 expression but its expression in high risk HPV infection was not appreciably increased. Shi et al have also opined that high risk HPV infection and Ki67 expression can be useful marker in the diagnosis and staging of CIN. A linear increase in Ki67 expression was also reported by Gatta et al from normal to dysplasia cases leading to cervical carcinoma. Ariana et al have found that Ki67 overexpression indicates HPV16 integration enhancing the chances of low grade SIL (LSIL) from the women with normal smears (40).

h) Claudin- 1 The claudin being blocking protein controls the cells signal transduction. Its overexpression has been found with increasing grades of SIL (Sobel, G et al). (41) Szabo, et al have found high expression of claudin1 in the precancer and frank cancer than in the normal smears and have suggested it as a useful diagnostic and prognostic marker in cervical carcinogenesis with therapeutical capability. (42) Attila et al have also lauded the claudin-1 as a new cervical cancer screening technique in minimizing the number of unnecessary conization and offering most cost effective clinical care.(43)

i) AgNOR PLEOMORPHISM AgNOR proteins are DNA loops controlling ribosomal synthesis and appear as black nuclear dots with sliver nitrate staining. The cell proliferation is reflected in the different configuration of AgNOR. The number of AgNOR dots have been used in the diagnosis of progression of cervical tumor. There are complex pleomorphic and single small dots in the nucleus which are counted separately in 100 cells to get mean count.

Since its introduction, the AgNOR technique has been used in finding out different neoplasm in biopsy and cytological materials, but its application in cervical cytology has been rarely employed. Darne et al have applied AgNOR technique for differentiating endocervical normal and adenocarcinoma and have found statistically significant difference in AgNOR counts between these two conditions.(44) Cardillo found AgNOR staining technique as discriminator between normal and metaplastic cells and between different grades of SIL.(45) Pratibha et al have found remarkable variation in AgNOR in different stages of cervical carcinogenesis (46).

AgNOR pleomorphism has been very useful in ascertaining the neoplastic progression of carcinoma cervix (Egan et al, Rowlands et al) (47,48). They have found number of AgNOR dots increased with the progression towards the malignancy. However, there is only single report to correlate HPV positivity with AgNOR pleomorphism which comes from Alarcon-Romero et al who have found association of AgNOR dots with high risk HPV types. The virus induces cellular alterations and abnormal AgNOR dots product of HPV activity and viral integration into human genome. They have suggested a correlation of both could be useful in ascertaining the progression of SIL to malignancy (49).

Recently, Srivastava A N et al have found a association of high AgNOR pleomorphic counts with presence of high risk HPV types and the SIL persistence (50). Hence, it can be used in differentiating SIL cases which have potential to develop into cervical carcinoma. This technique being simple and economical can be introduced in the place of costly HPV- DNA testing especially in a rural cervical cancer screening program.

j) NUCLEAR FACTOR KAPPA-B Since its discovery by Baltimore in 1988, NF-KB have been widely studied from Drosphila to man. It is found in the cytoplasma as active hetrodimers of IKBα and other units like p50 and RelA. This factor on translocation resulted in the initiation of development of cervical cancer through regulation of several genes.

Nair et al have been the first to demonstrate the activation this transcription factor during the development of carcinoma cervix and have found intense immunoreactivity in the cytoplasm in normal and LSIL samples but the nuclear positivity in the majority of HSIL and cervical cancer tissues (51). They have suggested this increase in nuclear positivity may be due to functional activation of NF-KB.

Bronco et al have occasionally found cytoplasmic NF-KB expression in CIN3 and squamous cell carcinoma cases and related to the high risk HPV detection and progression to CIN3 and cancer (52).

Li, et al have carried out immunohistochemical staining of NF-KB in different stages of development of cervical cancer and have found a gradual NF-KB activation with cancer development and progression.(53) They have studied p65 and p50 expression in the cervical tissues and have found minor NF-KB activation in normal and LSIL cases. However, this was elevated in the HSIL and cancer tissues and their nuclear expression levels were highly associated with disease development.

7) GENOTOXIC BIOMARKERS

Micronuclei are genotoxic biomarkers and are chromatin bodies in cytoplasm. They are accentric chromosome fragments left behind in the anaphase

stage of the mitotic cycle. These are feulgen specific bodies representing chromosomal damage. Countryman et al first reported these bodies in the peripheral blood lymphocytes and since then have been subject of study of epithelial carcinogens in different occupational settings (54). Harshwardhan et al have studied buccal scraping collected from patients with oral cancer and have found the frequency of micronuclei in cancer and precancer cases were four –fold and 3.87- fold higher than observed in other non-malignant pathology. (55) Samantha et al and Srivastava et al have studied micronuclei under pre-cancerous and malignant conditions of cervix and have found progressive increase in a micronuclei counts from normal to malignant conditions. (56)(57) This technique being simple with no cost as the counts can be done in the Pap smears examined, can easily be introduced in cervical cancer screening programs mainly in rural areas.

8) TELOMERASE ACTIVITY

A critical enzyme needed for cell growth- telomerase is activated in many cancers of human body. It becomes upregulated during progression in different cancers and has prognostic capability. Herbsleb, M et al have found increased activation of telomerase in cervical pre-cancer cases and its increased expression was seen in CIN 1 cases.

CONCLUSION

Nearly all the tumor markers mentioned in the article are though effective but are very expensive especially micro- RNAs and involve sophisticated techniques for the detection and hence are of only academic interest. In the Indian context, an economical tumor markers like AgNOR pleomorphic counts or micronuclei counts, the farmer being more reliable, could easily substitute the expensive HPV determination to find out high risk SIL cases in cervical cancer screening program mainly for rural population. This will help in minimizing the occurrence of cervical cancer in the rural population through adequate treatment and follow up. Further all the SIL cases will not be needed to be followed hence enhancing the efficacy of cervical cytology.

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