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MHC Class I Related Antigen A and B and NKG2D Receptor Expression in PAP smear: A Newer Paradigm of Diagnoses in Cervical Cancer

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Abstract

Cervical cancer (CC) is the leading second most common cancer in India in the past two decades. It has been seen that early detection of CC can lead to curing of diseases successfully. There is a pressing need to establish an effective biomarker that would precisely detect early CC. Recently lots of attention has been passed on Major histocompatibility complex class I related chain A and B (MICA/B) antigens. This molecule is stress-inducible ligand that is over expressed in several cancers. However presence of this antigen is not yet been studied in cervical cancer (CC) patients. The present research studies for the first time expression of MICA/B antigen on PAP smear samples of CC patients. The PAP smear samples of control and CC patients were analyzed by Haematoxylin and eosin staining (H&E), MICA/B Immunoflurocence staining and double-immunofluorescence staining with anti-MICA/B and NKG2D antibody. Our results are highly motivating and clearly suggest the increased presence of MICA/B and decreased NKG2D receptor expression molecules. This decreased NKG2D expression might result in immune escape and disease progression. So far there is no data is available of any such studies on relationship between expression : of MICA/B in PAP smear of cervical cancer patients.

Introduction

Cervical cancer (CC) is the leading second most common cancer in India in the past two decades. Its multiple causes, prevention potential make cervical cancer an important disease for in-depth studies [1,2]. High risk women such as multiple sexual partners, multiple pregnancies, poor genital hygiene, malnutrition, use of oral contraceptives, and lack of awareness, commercial sex workers, Specific types of oncogenic HPV-16, 18 and HIV (human immunodeficiency virus) positive women are more prone [3]. Incidence of cervical cancer is more in 55-59 years and a considerable population report in the late stages of disease. Hence, there is a pressing need to establish an effective biomarker that would precisely detect early CC. Cervical intraepithelial neoplasia (CIN) is a precancerous lesion if diagnosed, can be treated effectively to prevent progression to cervical cancer. It well understood that the cells under stress will over express Major histocompatibility complex class I related chain A and B (MICA/B) antigens [4,5]. MICA is a stress-inducible ligands that bind to the immunoreceptor NKG2D and play an important role in mediating cytotoxicity of NK and T cells [6,7]. These are the molecules up regulated in response to various stimuli of cellular stress including heat shock, infection with viruses, malignant transformation and inflammation.

Expression of MICA/B is increased in several malignancies such as Oral, Cervix, and Breast Cancer [8]. There is no data available on expression of MICA/B in Pap smear. In this study, we propose to study immunohistochemical expression of MICA/B as potential biomarkers for the detection of early stage of CC in Pap smear to indicate CC progression. MICA/B will be a biomarker for early detection or monitoring. Improvements in CC diagnosis, monitoring and response are immense need of CC research. New approach of diagnosing CC by Pap smear will be a non invasive technique that will help to decide the treatment outcome future direction. So far there is no data is available of any such studies on relationship between expression of MICA/B in pap smears of CC. This screening test will be a cheap, effective

that can help to detect disease early and may reduce the burden of skyrocketing costs.

Materials and Methods

The use of specimens from human subjects is approved by the Institutional Review Board of DY Patil University. Provided informed consent of patients was taken. Patients (n= 10) having age group 25 to 50 years of age and an expected survival of at least 1 year are involved in this study. Patients having any serious illness, infection, psychiatric illness, pregnant, nursing woman were excluded from the study. Pap smears from healthy females (n=5) were used as contols. Pap smears of proven CC patients were collected from D Y Patil hospital, Kolhapur and Shri Siddhiviyak Ganpati cancer Hospital, Miraj. The cells were collected from the outer opening of the cervix at the transformation zone where the outer squamous cervical cells meet the inner glandular endocervical cells. Smears were taken on the positively charged slides (pathnsitu biotech). Pap smears of both control and Cancer cervix were fixed with chilled acetone-methanol (1:1), air dried and kept at -40ºC. Before processing, slides were washed with D/W containing 0.05% tween 20. Serum blocking was done by goat serum and slides were incubated with PE conjugate MICA/B (Molecular Probes, USA) for one hour at room temperature followed by wash with D/W containing 0.05% tween 20. Double staining was done using MICA/B (Alexa 488) and NKG2D (Alexa 594). Slides were mounted with DAKO mounting media.

Results and Discussion

Cervical cancer is the most common malignancy particularly in India. Nowadays, cervical screening is necessary in every woman above 40 yrs because cervical precancers do not show any signs and symptoms at its early stage. In India, even though a major effort is taken to expand cytology services, then also it is not be possible to screen even one-fourth of the population in the near future. Due to lack of awareness regarding cervical cancer in women and failure to regular screening, the carcinoma cells undergo invasive phase and develops CIN progression. These women are more prone to invasive cancer in the future. The focus must be emphasized on detection, prevention and cure of cervical cancer in a highly populated country like India to prevent its extensive spread. The early detection will definitely help to reduce the morbidity and mortality of CC. Most of the time disease gets diagnosed in late stage and patient have to undergo radiation and brachy therapy. Because of the side effects of the therapy, it is important to detect the CC in its early stage and to reduce the side effects and high risk of patients

HE staining of control slide showed many sheets of glandular cells. The sheets were tightly crowded but lacked feathering at the edges (Figure 1A). Slides of CC showed a disrupted, irregular and honeycomb like pattern of thickened cell borders and overlapping nuclei (Figure 1B). MICA/B that binds to the immunoreceptor NKG2D mediates cytotoxicity of NK and T cells. When MIC molecules release from the cell surface, they escape from immune recognition and responsible to form tumor cells and thus an aggressive tumor growth. Expression of MICA/B is increased in several types of malignancies. NK cells defense against viral pathogens via cytokines and chemokines secretion and kill infected cells. NK cells plays crucial role in tumor immunosurveillance. Low levels of expression of MICA/B in the pap smears of control (Figure 2A) was observed. Expression in CC patient was strong in glandular cells (Figure 2B).

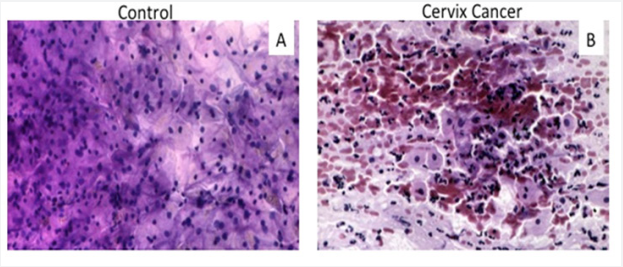

Figure 1: Haematoxylin and eosin staining of PAP smear.

A.Control showed sheets of glandular cells,

B.Cervical cancer patient showed disrupted, irregular and honeycomb like pattern.

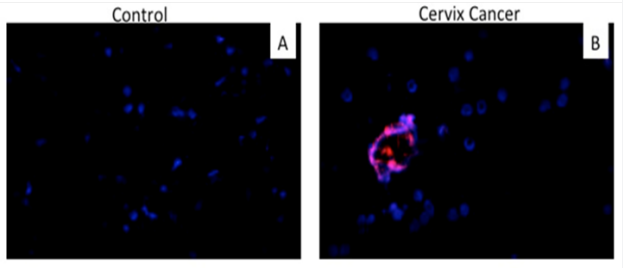

Figure 2: MICA/B Immunoflurocence staining of PAP smear.

A.Low levels of expression of MICA/B in cells.

B.Cervical cancer patient showed intense expression of MICA/B (Red) in glandular cells.

(Microscope field at a magnification of X40).

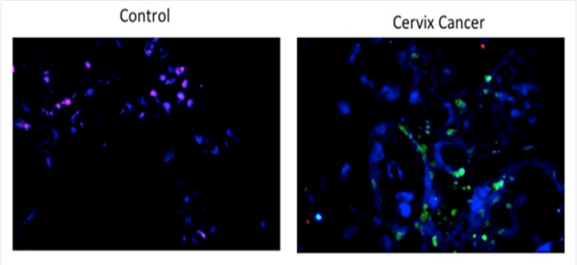

Figure 3:

A.Merged images revealed that control patient showed NKG2D+ cells (Red).

B.Cervical patient showed Strong MICA/B expression (Green) while NKG2D (Red) was very less.

(Microscope field at a magnification of X40).

Recently, it has been stated that activating NK cell receptor ligands MICA (NKG2D ligand) are differentially expressed during the progression to CC [9,10]. The aim of the present

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work was to study MICA/B and NKG2D expression in patients with CC. Expression of NKG2D in control sides was moderate (Figure 3A) However, decreased expression was observed in CC slides (Figure 3B). NKG2D is constitutively expressed on NK and T cells to mediate recognition and destruction of MIC A/B-expressing cells. Decreased NKG2D expression might result in immune ecape. The role of MICA/B and NKG2D is well studied in tumors and its possible role in tumor immune recognition or suppression but data regarding its study in Oral, Cervix, and Breast cancer are very few. In this study, immunohistochemical expression of MICA/B + NKG2D receptor on Pap smear were studied as potential biomarkers to indicate cervical cancer disease progression. However, the expression of triggering receptors MICA/B + NKG2D from patients with cervical cancer remains unknown.

Conclusion

In summary, the present research clearly suggests that the increased presence of MICA/B and decreased NKG2D receptor expression molecules in Cervical cancer (CC) patients for the first time. This decreased NKG2D expression might result in immune escape and diseases development. The results are highly encouragering and have immense potential to use in clinical setting. These results can be clinically correlated and can be used to predict the diseases progression and helps clinician to start appropriate therapy. Tough the sample size used in the research is comparatively small but the available results are giant leap in cervical cancer research. Our lab in D.Y. Patil University is continually working on the expression of this molecule in oral and breast and critically correlating the clinical correlation and near future we may come out with some more existing research.

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