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ROLE OF KI-67 AS A DIAGNOSTIC TOOL IN ORAL SQUAMOUS CELL CARCINOMA-A REVIEW

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ABSTRACT

There are many cell-cycle regulators but Ki-67 remains one of the most important antigen. Immunohistochemistry determines the expression of Ki-67 antigen. In all active phases of the cell cycle, there is a nuclear non-histone protein of 395 KD which is expressed and reacts with the anti- body Ki-67. In several studies Ki-67 is considered a prognostic factor for breast cancer. A high Ki-67 labelling index is associated with poor differentiation of tumors and large tumor size in breast carcinoma, carcinoma of the tongue, which were demonstrated in many studies. Many studies stated that in oral squamous cell carcinoma Ki-67 can be considered a good prognostic marker.

Keywords: KI-67, Oral Squamous Cell, Carcinoma, Immunohistochemistry

INTRODUCTION

The tumor proliferation rate is often compared and correlated with prognosis. This marker is evaluated through immunohistochemistry. The proliferative

rates of tumor have been evaluated in attempt to correlate them with prognosis **[1]**. Immuno histo chemical antibodies are directed against the Ki-67 antigen. The tumor's aggressive behavior is reflected by the expression of Ki-67 antigen. Here, the patients of high risk are taken into consideration. The expression of the Ki-67 is based on the percentage of tumor cells that stain positively by the antibody **[2]**. The Ki-67 is recognized by monoclonal antibody MIB-1 in the formalin fixed paraffin embedded tissue sections **[3]**. The study of Norbury and Nurse in 1992 and Kirschner in 1992 found some other markers such as p34-2 or p53 which involve in the modulation of cell cycle.

The prognosis of the tumor is best correlated with the following criteria, namely immunohistochemical analysis in which the antibodies are directed against the Ki-67 antigen, cytometric S-phase fraction(SPF), proliferating cell nuclear antigen, thymidine labelling index and mitotic index **[4]**. Metastasis is another important criteria in which prognosis can be evaluated **[5]**.

Characteristics of PKI67

In 1980, the Ki67 antigen was originally identified as a proliferation-associated nuclear antigen, which was identified in cell division, G1-, S-, G2- and M-phase, while GO phase doesn't exhibit the antigen **[6]**. G1 and S phase show low levels of Ki-67 and elevated levels in early mitosis. There will be abrupt decrease in Ki67 in latter phases of mitosis **[7]**. The Ki67 gene encoding is a continuous sequence of 29,965-bp length located on chromosome 10q25 and 15 exons with sizes 67-6845bp and 14 introns with sizes 87-3569 bp. Exon 13 contains 16 homologous segments of 366bp (Ki67 repeats) located at the center of this gene. The gene in Ki67 protein is comprised of a 74bp 5' region and a 264 bp 3 region **[8]**. In the cell cycle, the presence of Ki67 at any time is con- trolled by an exact balance between the synthesis and degradation, indicated as 1-1.5 h of its short half- life **[9]**. The expressions of the structure are regulated by proteolytic pathways, also controlled by the key regulatory complex cyclin -dependent kinase-2 **[10]**. The other proteins such as DUN1 and RAD are associated with Ki-67 in the cell cycle regulation **[11]**. For assuming gene transcription, a stamping tool of Ki67 promotor region is needed **[12]**. The Sp-1 binding site is important for the transcriptional regulation of the Ki67 gene **[13]**. There is a correlation between p53 and ki67 in many types of cancers including oral squamous cell carcinoma and breast cancer **[14]**. The percentage of tumor cells stained by antibody remains

the foundation for scoring system **[15]**.

Scoring Methodology

In order to evaluate the methodology of the study, a publication is scored utilizing the World Health Organization's classification system **[16]**. The scientific design and the laboratory method description assist in detecting the presence of Ki-67, DNA/RNA or antibodies against Ki-67 **[17]**. Bright field microscope is used for this Ki-67 immunostaining **[18]**. Many clinical laboratories investigated the use of Ki67 as a diagnostic tool and obtained successful results **[19]**. The immunostaining remains the gold standard for determining the expression ofKki67. With the cutoff between 10-14%, positively -stained cells are interpreted as high risk in terms of prognosis **[20]**. In hormone related breast cancer the Ki67 labelling index played a key role in endocrine therapy in addition to chemotherapy and this was discovered by St Gallen Consensus in 2009. The labelling index of Ki67 in tumors are graded as low, intermediate and high based on the following scores, ≤15%, 16%–30%, and >30%.

The tissue microarray technology has become popular and convenient for evaluating different kinds of tumors **[21]**. This technology is also helpful in identifying cancer related genes **[22]**.

PKI67 as a Diagnostic Tool

The cell proliferation in malignancies can be best deducted by Ki67 **[23]**. The expression of Ki67 in malignancy is high when compared to normal tissue **[24]**. As the Ki67 expression increases there will be decrease in tissue differentiation also which highly correlates with metastasis and the clinical stages of tumor **[25]**. The tumor proliferation rate can be assessed through the following criteria, namely flow-cytometry, mitotic counting, synthesis phase fraction and immunohistochemistry. The commonly used monoclonal antibodies are Ki67/Mib-1 which are expressed in phases of G1, S, G2 and M in cell cycle, but not in resting phase **[26]**. Ki67/Mib-1 is associated with grading system.

[27] As the presence of Ki67 is dominated in a variety of tumors, it can be correlated with the tumor staging system **[28]**. The combination of proliferative marker and histopathological examination are useful to identify the recurrence **[29]**. Early diagnosis can be obtained by deduction of Ki67 expression whereas the conventional diagnosis of the past decade was done only in the advanced stage of malignancy **[30]**. Along with Ki67, bcl2, p53, PCNA and CD105 have been investigated. In severe pancreatic tumor, the Ki-67 and smac gene have a close

association leading to better diagnosis, prognosis and treatment **[31]**. Based on this, the efficiency of conventional grading system is en- hanced by novel molecular grading system **[32-35]**. The expression of Ki67 is higher in Duke stage 'B' carcinoma when compared to Duke stage 'C' carcinoma.

The Ki-67 expression is low in poorly differentiated adenocarcinoma and mucinous carcinoma, whereas it is high in well and mod- erate adenocarcinoma. Hence it has been suggested that the proliferation rate is low in poorly dif- ferentiated carcinoma. The fixation resistance of the epitope of Ki67 antigen is recognized by MIB- 1 expression **[36]**. In many fields of pathology the Ki-67 index serves as a diagnostic and prognostic support tool. **[37]**. The estimation of Ki67 index is inconvenient and time consuming coupled with subjective to inter-observer inconsistency **[38]**. The use of an IHC cocktail improves the accuracy of Ki-67 index and this was recommended by recent research. Here the T-cell(MART-1) recognizes Ki-67, melanocytic marker, and melanoma antigen **[39]**. The present research supports the usage of Ki-67/MALT1 which brings out accurate distinguishing characters of melanocytic cells from lymphocytes, stromal cells and epithelial cells with Ki-67 positivity in proliferative cells in the field of melanocytic pathology **[40]**.

PKI67 as a Prognostic Tool

The Ki-67 is expressed in all cell cycle phases, except the resting or Go phase. Its use as a prognostic marker over mitotic rate is recommended by Academics**[41]**. There exists a correlation between proliferative markers and grades of tumor **[42]**. This study also suggests that the Ki-67 expression is useful for choosing a specific drug regime and proves to be an independent prognostic marker for survival rates which includes all staging and gradings **[43]**. The correlation between the Ki-67 malignant cells and the survival rates of the patient have been demonstrated in this study. The pre-re- medial evaluation of Ki-67 expression has become more significant in demonstrating tumor aggressiveness and for the selection of appropriate treatment **[44]**. In a variety of malignancies the intense immunohistochemical staining reveals poor prognosis **[45]**. In one study, the scores were reported as 40 for Ki-67 and 80 for PCNA approximately which was seen in anorectal malignant melanoma, but in cutaneous malignant melanoma it was seen to have a better prognosis than anorectal malignant melanoma **[46]**. The

negative correlation of Ki-67 is indicated as the best diagnostic and prognostic tool for mucosal malignant melanoma [MMM]. Hence these studies indicated that Ki-67 antigens are useful for targeted therapies, protein labelling index and cell proliferation. The com- bined network of antisense oligonucleotide and Ki-67 antigen is responsible for tumor growth inhi- bition. Anti-Ki-67 PNAs produce a robust inhibitive effect on Ki-67 expression than ASOs, and have considerable effects on the proliferation and apoptosis of human renal carcinoma cells **[47]**. Short heparin RNAs is an another important criteria for diagnosing the cell proliferation **[48]**. An- other study mentioned about the oncolytic adenovirus sh RNA expression which has the lytic ability on tumor proliferation and also mentioned about the delivery of Ki-67-sh RNA expression **[48]**. Moreover the cancer proliferation can be controlled by suppressing the Ki-67 activity through microinjection of antibodies which is directed against the Ki-67 antigen. Recently non-cationic pho- to immunoconjugated encapsulating liposomes[PICELs] introduced by Zhang *et al* were found to play effective roles against the cell proliferation. Hopefully the moderate successful cancer treatment is based on the following criteria namely molecular etiopathogenesis and molecular targeted therapies **[48]**.

CONCLUSION

In the field of pathology, the cancer proliferation rate can also be assessed through the strong ex- pression of Ki67. It is extensively used as a prognostic marker. Ki67 plays a significant role in mol- ecular pathology that in turn create a pathway for better diagnosis and treatment (modified)

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