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Review Article

Role of Ki67 protein in colorectal cancer

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ABSTRACT

Colorectal cancer has become one of the most frequent malignancy and the leading cause of death from cancer globally. Global Burden Cancer in 2013 had placed colorectal cancer in third place morbidity level and forth most lethal cancer globally. Understanding prognostic marker is essential to increase prognosis prediction in patients with colorectal cancer, especially in patient with the later stages. Several studies have reported the prognostic value of Ki67 expression in patient with colorectal cancer. Ki67 protein expression is associated with proliferative activation from intrinsic cell population in malignant tumor cells, where Ki67 can be used as a marker for tumor aggressivity. Expressions from protein Ki67 are associated with proliferative activities from intrinsic cell population in malignant tumor cells, where Ki67 can be used as marker from tumor cell's aggressivity. Several diagnostic applications for pKi67 has already been explained, where Ki67 is significantly higher in malignancy compared to normal tissue. pKi67 is also increased along with the decrease of tissue differentiation, and it correlates with the presence of metastasis that can not be seen and tumor clinical phase. Prognostic value from Ki67 had been investigated in many studies with their potential as reliable marker in breasts, soft tissue, lungs, prostate, cervix, and central nervous system. Positive expression from Ki67 in colorectal cancer shows a better prognosis in patient who received surgical treatment and adjuvant radiochemotherapy, but not in a patient who received only a surgical treatment.

Keywords: Colorectal cancer, Diagnostic, Expression of protein, Function, Ki67 protein, Prognostic

INTRODUCTION

The origin of Ki67 protein was first defined as the prototype of Ki67 monoclonal antibody, which was firstly developed from immunizing mice with nuclei from L428 lymphoma Hodgkin cells. The name was derived from the city where this protein was found, which was from Kiel in Germany. Ki67 antigen is an antigen which codes two isoformed proteins with molecular weight of 345 and 395 kDa. It was initially identified by Scholzer and Gerdes in the early 80s.[1] Ki67 protein has 1 to 1.5 hours of half-life. Ki67 was found in every cell cycle's active phase (G1, S, G2, and M), but it was not found in resting phase (G0). In the late phase of mitosis (while anaphase and telophase), there is a significant reduction of Ki67 protein level.[2] Ki67 protein expression is associated with proliferative activation from intrinsic cell population in malignant tumor cells, where Ki67 can be used as a marker for tumor aggressivity.[3] Prognostic value from Ki67 had been investigated in many studies with their potential as reliable marker in breasts, soft tissue, lungs, prostate, cervix, and central nervous system.[4,5]

REVIEW OF LITERATURE

Nowadays classification scheme might need revision, where biological activity and prognostic significance from these tumors are in attention. There is significant rise in number of studies which showed Ki67 is an

important factor in cancer grading and evaluate the prognosis. It was proven that immunohistochemistry staining (IHC) is the effective method in determining the prognosis in several tumor types (Figure 1).[6,7]

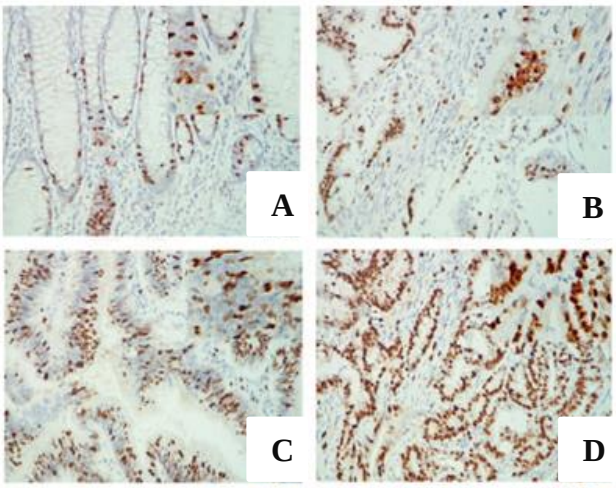

Figure 1: Immunostaining of Ki67 40%. A), 60%, B) 70%, C) 80%, D).[6]

Colorectal cancer has become one of the most frequent malignancy and the leading cause of death from cancer globally. Global burden cancer in 2013 had placed colorectal cancer in third place morbidity level and forth most lethal cancer globally. In 2013, there was 1.6 millions incidents of colorectal cancer and 771 000 deaths.[8] Moreover, the incident number shows a significant rise. Surgery with combination of adjuvant radiochemotherapy are the main treatment of colorectal cancer. Although 5-year survival rate in the early stages of this cancer can as high as 90%, the prognosis in the late stages are still poor.[9]

Therefore, understanding prognostic marker is essential to increase prognosis prediction in patients with colorectal cancer, especially in patient with the later stages. Several studies have reported the prognostic value of Ki67 expression in patient with colorectal cancer. Prognostic value in Ki67 expression showed a poor survival in patient with breast cancer, glioma, cervix cancer and hepatocellular carcinoma. But the prognostic value of Ki67 in patient with colorectal cancer is still a controversy.[10]

Characteristics of Ki67

Antigen of Ki67 was first identified in 1980, as a nuclear antigen which was associated with proliferation (Figure 2). It can only detect proliferating cells (phase G1-, S-, G2, and M) and can not be detected in resting phase (phase G0). The level of Ki67 is low on phase G1 and S, and highest at mitosis. In the late phase of mitosis, the Ki67 level is declining drastically. Gene, which coded Ki67, is a continue sequence with 29,965-bp in length which is located in chromosome 10q25-ter and consists of 15 exons with size range from 87 to 3569 bp. Exon 13 has 16 homolog segments from 355 bp (Ki67 repeats) which is located in the middle of this gene. The complete gene consists of 74bp 5th region and 264 bp 3rd region in Ki67 protein.[11,12]

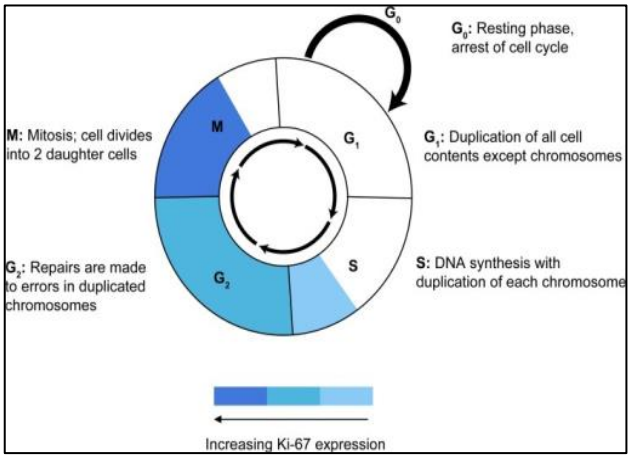

Figure 2: Ki67 in a cell cycle.[13]

The amount of pKi67 in any cell cycle are regulated with precise balance between synthesis and degradation, which was indicated in a short half-life, 1 to 1.5 hours. Ki67 protein expression coincides with cell transits during mitosis and experiences phosphorilations and dephosphorilations during in vivo mitosis, which gave a weak point in degrading protease. The structure of Ki67 indicates that its expression is regulated by proteolytic path, which is controlled by a complex regulator key cyclin B/cyclin-dependent kinase 2. The pKi67 was known to have structure resemblances (including those which is called as association of fork head domain) with another protein such as DUN1 and RAD, which was also linked with the regulation of cell cycle.[7,14]

The characteristics of Ki67 promotor region becomes an essential understanding from gene transcription, so that is important to investigate this matter to expand the interventional target which can modulate gene expression. In the previous study, *deletion* analysis and *lucipherase* dual reporter assay were used to localize the core from Ki67's promotor from -223 to +12 nt relative to the location of transitional inisiation, where there are fewer TATA< and region such as GC consist of several Sp1 binding site. It has been demonstrated that region from -223 to +12 nt can do a transcription from gene Ki07 and Sp1 binding site was essential in regulating transcriptional from Ki067 gene. Electrophoretic mobility shift assay shows three Sp-1 binding sites in Ki67 promotor, which was essential to transcriptional basal activity.[15]

It was found that expression from p53 has been correlated with Ki67 in several type of cancer, for example in oral squamous cell cancer, and breast cancer. How p53 can influence gene Ki67 expressions is still unclear. When

there are three Sp-1 binding site in Ki67 promotor, p53 are also suppressing gene transcription in Sp-1 binding site's promotor. It was estimated that p53 halts Ki67 promotor activities through p53 pathway and sp-1 dependent. It was hypnotized that at least there are 2 regulator transcriptional mechanism. The first one is p53-binding motifs which influence suppression of promotor Ki67 transcriptions. The second is a possibility of interactions between p53 and Sp-1 in Sp-1 binding site on Ki67 promotor.[15]

Older age is associated with elevation of Ki67, where aging is a process that cannot be altered, and cancer is a disease which are more frequently seen in elderly. Aging and cancer have many similarities such as genome instabilities, although cancer cells are often benefited from mutation, other cell accumulates mutation damage, resulting in physiology decline and aging. Telomere attrition is also a common characteristics, but cancer cells can avoid cell cycle defenders by activating telomerase enzyme. Inhibitor enzyme which is responsible in cell's proliferation can also experience kovalem modification against DNA and histone. Proteostasis is disturbed in aging and cancer cells, protein accumulative aggregations and toxic effect were general characteristics of several disease which linked with age. Cancer avoid this process with regulating chaperone activity, proteasome, and lysosome.[16]

Scoring and methods

Scoring systems are based by cell tumor percentage which was stained with antibodies. To use the methods from this study, each publication is scored with the classifications system from World Health Organization (WHO). Scores are determined from several aspects from methods, which has grouped into three main categories. Scientific design, description from laboratory methods which was used to identify the presence of pKi67, DNA, RNA or antibodies for Ki67, and several clinical reports have used this techniques to detect Ki67.[17]

Briefly, automated bright-field microscope, and softwares are used to detect and classify. After staining of Ki67 slide was observed with 5 times magnifications, one clinical laboratory experts, who does not know the histology diagnosis and patient safety data, randomly selected for at least 8 field of view which represent the distance from Ki67 immunostaining in tumor that previously was isolated to be evaluated with automated bright-field microscope in 20 times magnification.[18]

DISCUSSION

pKi67 as diagnostic tools

Ki67 was frequently used as indicator in proliferating cells.[19] Several diagnostic applications for pKi67 has already been explained, where Ki67 is significantly higher in malignancy compared to normal tissue. pKi67 is also increased along with the decrease of tissue differentiation, and it correlates with the presence of metastasis that can not be seen and tumor clinical phase. Proliferation activity in tumor can be set with calculating the mitosis, determine cytometric-flow in fraction of synthesis phase and immunohistochemistry with using reactive antibody against several cellular proliferating antigen. Ki67 monoclonal antibody/MIB-1 generally used and reacts with Ki67 nuclear antigen that emerge while cycle cells phase G1, S, G2 and M, but can't be found during G0. Percentage of immunoreactive tumor core cells pronounced as index labelling (LI) studies today showed positive correlation between Ki67/MIB-1 LI and tumor stages in human malignancy. Due to limitations of routine histology exams in tumor for predicting tumor's behaviour, immunostaining of Ki67/MIB-1 has been introduced due to their potential to elevate information's regarding scoring systems.[20] Their presences in several types of tumor show that it is possible to use Ki67 in routine scoring of cancer. Wise usage of this proliferating markers with the combination of histopathology study from malignancy can be used as a more reliable indicator regarding the possibility of tumor relapse.

Data regarding Ki67 as diagnostic markers are rare and according to several laboratory methods and statistics. Cancer have complex pathogenesis and initial diagnosis are hard to rely on until the disease has evolved into late stages. Therefore, further study regarding diagnostic and prognostic markers can help initial diagnosis. Especially, Ki67 expression shows tumor's proliferation activity and correlated with initiation, evolution, metastasis and prognosis from several types of tumor. Particular regulators from this process, such as Smac maintenance mini 7 chromosomes (77), p53(10,78-80), Bcl-2, proliferates nuclear cell antigen (PCNA) and CD105, have been studied.[21]

In several experiments, Ki67 seems to correlate strongly with pancreas tumor severity and with Smac expression, it is probably useful as diagnostic and prognostic markers, or, in their relationship with Smac, can be used as treatment efficacy indicator. In further study, Chen and colleagues report that using Ki67 LI and vascular endothelial growth scoring are useful to effectively and accurately predict the result and optimize the treatment in evaluating invasive bladder cancer non muscular. This new molecular grading system can increase the efficiency of conventional system.[22]

Colorectal carcinoma is the 4th main cause of cancer related deaths all around the world. It was shown that Ki67 LI was higher in Dukes' stage B than in Dukes' stage C. It was concluded that Ki67 is positive in poorly differentiated adenocarcinoma and mucinous carcinoma are significantly lower if compared with well differentiated adenocarcinoma and moderately differentiated, which shows that proliferative activity is lower in cancer with poor differentiated tumor. But in

Dukes early stage (A or B) adenocarcinoma non-mucinous shows a high level of Ki67.

pKi67 as prognostic tools

It was known that Ki67 was expressed in every cell cycle outside resting phase G0. Experts recommend their usage as a prognostic marker for mitosis level. Several studies have shown the correlation between proliferative markers and tumor grading. Several studies have also shown their usage as prognostic markers and have presented in a patient, who had already given specific regiment therapy based on grading of pKi67 expression. Whereas prognostic biomarker indicates the possible way for a disease in an individual who did not receive therapy, a predictive biomarker identifies subpopulation of patient with a high chance of giving response from a therapy. The result from one study indicated that Ki67 label index are an independent prognostic factors for safety level, including all stadium category and grading. This study demonstrates correlation between ratio of Ki67 in positive malignant cells and patient safety.[23] Vogt and Klapper showed that Ki67 index were associated with patient's prognosis with later stage cancer.[24]

Prognostic value of Ki67 expression in patient with colorectal cancer is not consistent. Investigation which were done by Weber showed that a positive Ki67 expression indicates a decline safety level in colorectal cancer patient with metastasis into liver.[25] Handa also analysed the prognostic significances from Ki67 expression, but found that this biomarker is not significant in colorectal cancer cases.[26] Other study also showed Ki67 expression in 412 colorectal cancer patients,[27] and found that patient with high Ki67 are linked with better outcome in patient who received a surgical treatment, combined with adjuvant chemotherapy, but not in patient who only received a surgical treatment.[23,28] The writer explained that cancer cells which proliferates quickly can be susceptible from cancer cell death, induced by chemotherapy. Itamochi also explained that low proliferation in tumor cell can contribute to resistance against chemotherapy in a patient with renal clear cell carcinoma.[29]

In a metaanalysis study, positive Ki67 expression in colorectal cancer shows a good prognosis in a patient who received surgical treatment and adjuvant radiochemotherapy, but not in a patient who only received a surgical treatment only. The possible explanation regarding contradictive impact by Ki67 expression on prognostic value in colorectal cancer patient with higher proliferation level is due to more responsive from radiochemotherapy. Mechanism of radiochemotherapy in a cancer therapy is to eliminate several specific cells, especially quick proliferating cells. And evaluation of Ki67 expression in cancerous tissue often shows that cell is in a high proliferative activity and the probability of cell getting killed is also higher. Adjuvant radiotherapy can eliminate fast proliferating cells in rectal cancer. It can be concluded that high Ki78 immunostaining can give impression of increase in radiochemotherapy response and indicates a better prognosis in patient with colorectal cancer who received surgical and adjuvant therapy.[27] Nevertheless, it is still difficult to set the cut-off points from Ki67 expression. Fluge concluded that increase in protein Ki67 expression more than 40% in a tumor cell is associated with better safety levels without relapse from colon cancer stadium 2 and 3, but not in rectal cancer. In addition, in a stadium 3 colon cancer, high level of Ki67 (>40%) are a predictive marker to evaluate the effects of adjuvant chemotherapy.[27]

CONCLUSION

Expressions from protein Ki67 are associated with proliferative activities from intrinsic cell population in malignant tumor cells, where Ki67 can be used as marker from tumor cell's aggressivity. Monoclonal antibodies Ki67/MIB-1 usually used and are reactive to Ki67 nuclear antigen that emerge in cycle cell phase G1, S, G2 and M but they are not found in phase G0. Positive expression from Ki67 in colorectal cancer shows a better prognosis in patient who received surgical treatment and adjuvant radiochemotherapy, but not in a patient who received only a surgical treatment.

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