

The Correlation of Autophagy and Glycolysis Mechanism to The Histopathological Grading of Penile Squamous Cell Carcinoma Through Expression Microtubule-associated Protein 1 Light Chain 3B (LC3B) and Glucose Transporter-1 (GLUT-1)

Riski Hidayat^a, Jessy Chrestella^b, Lidya Imelda Laksmi^c

*Department of Anatomik Pathology, Faculty of Medicine,
Universitas Sumatera Utara, Medan, Indonesia
riski.pa.usu.19@gmail.com*

ABSTRACT

Background: Penile cancer incidence is still common in developing countries. Squamous cell carcinoma (SCC) is the dominant histopathological type found in penile cancer. Autophagy and glycolysis play important roles in cancer pathology. LC3B is one of the most widely used autophagy markers and GLUT-1 is an isoform of the glucose transporter family that is expressed in many cancers.

Objective: To analyze correlation of autophagy and glycolysis mechanism to the histopathological grading of penile squamous cell carcinoma through expression microtubule-associated protein1 light chain 3B (LC3B) and glucose transporter-1 (GLUT-1).

Method: This study is an analysis with a cross sectional approach on 50 samples of paraffin blocks diagnosed as penile squamous cell carcinoma. All samples met the inclusion criteria and were reviewed by 2 pathologists. The results of the immunohistochemical expression of LC3B and GLUT-1 will be assessed for correlation with the histopathological grade of penile SCC. The statistical test used in this study was the Mann Whitney test.

Results: A total of 50 samples of penile SCC, obtained grade I as many as 17 samples (34%), grade II 22 samples (44%), and grade III 11 samples (22%). From the results of the immunohistochemical expression of LC3B and GLUT-1 on the histopathological grading of penile SCC, the same results were found. In grade I, 15 samples were positively expressed, and 2 samples were negative. In grade II, all samples were positively expressed, and in grade III, 9 samples were positively expressed and 2 were negative.

Conclusion: There was no significant correlation between the histopathological grading of penile SCC with the immunohistochemical expression of LC3B and GLUT-1 ($p=0.818$).

Keywords: penile cancer, autophagy, glycolysis, LC3B, GLUT-1

1. Introduction

Penile cancer is a rare cancer, with a prevalence of 0.1-1 per 100,000 men in high-income countries and in countries where the majority of the population is circumcised.^{1,2,3} However, in less developed countries, including some countries in South America, Asia, and Africa, the incidence is very high. Brazil, Canada, and Romania are countries with the highest incidence of penile cancer in the world, with a prevalence of 6.1 per 100.00 men.^{2,3} Data from the Global Burden of Cancer (GLOBOCAN) 2018 shows 34.475 new cases of penile cancer in the world and 15.138 deaths related to penile cancer.^{2,4}

Squamous cell carcinoma (SCC) of the penis is the predominant histopathological type of all penile cancer.^{4,5} Although the pathogenesis of penile SCC remains unclear, previous studies have identified many potential risk factors, including phimosis, human papilloma virus (HPV) infection,

human immunodeficiency virus (HIV), balanitis, and multiple sexual partners. Meanwhile, tobacco use, age and race may also be associated with the development of penile SCC.^{4,6}

According to Hanahan and Weinberg, tumor cells exhibit eight special characteristics, which are referred to as hallmarks of cancer, which include sustained proliferation, evasion of growth suppressing signals, replicative immortality, angiogenesis, immune escape, evasion of cell death, metabolic reprogramming and activation of invasion and metastasis. . Recent reports suggest that autophagy is associated with some of these markers. For example, autophagy and apoptosis are usually thought of as opposing pathways, but under certain biological circumstances, they act cooperatively for cell death.⁷

Autophagy is an important cellular process that generally protects cells and organisms from unfavorable environments, including limited availability of adenosine triphosphate (ATP) or deficiency of essential nutrients. Metabolic reprogramming, which is one of the hallmarks of cancer, has recently attracted interest in the field of cancer therapy. It is known that cancer cells prefer to use glycolysis rather than oxidative phosphorylation (OXPHOS) as their main energy source to produce ATP rapidly even in an aerobic environment which is called the Warburg effect. Both autophagy and glycolysis play important roles in the pathological process of cancer. The mechanism of metabolic alterations to promote tumor development is its ability to regulate autophagy.⁸

In addition, numerous studies have shown that autophagy and glycolysis have been associated with cancer initiation and cancer therapy for several years. Some studies have shown that autophagy is a regulator of many oncogenes and tumor suppressor genes, whereas other studies have shown that autophagy is involved in the promotion of tumorigenesis and the development and inhibition of cancer.⁹

Microtubule-associated protein 1A/1B-light chain 3B (LC3B) is the most widely used marker for monitoring autophagy. Lipidated LC3B (LC3B-II) is incorporated into the outer and inner autophagosomal membranes, and thus makes it possible to detect different stages of autophagic vesicles. A growing body of research is finding that autophagy can be highly selective. Selectivity is achieved by autophagy receptors which on the one hand are able to recognize ligands and on the other hand interact with autophagy machinery.¹⁰

High glycolytic activity cannot be said if it is not accompanied by an increase in glucose uptake. Of all the glucose transporters (GLUT), GLUT-1 is the isoform that is most expressed in cancer cells such as breast, lung, colorectal, prostate, and hepatocellular carcinomas. Overexpression of GLUT-1 has been associated with tumor aggressiveness and poor survival in several cancers. Hypoxic Inducible Factor-1 (HIF-1) increases the expression of GLUT-1 which allows a higher capacity for increased glycolysis. In addition, the proto-oncogene c-Myelocytomatosis (c-Myc) increases GLUT-1 expression in cancer cells. Mutations of the p53 gene result in dysregulation of the GLUT transporter that increases its expression in cancer which contributes to facilitating glucose utilization in cancer cells.^{11,12}

The prognosis for penile SCC is generally determined by stage. However, there are several other additional factors that have a role in the prognosis of these tumors, namely tumor grade, presence of lymphovascular invasion and perineural invasion, size of the lymph nodes that have metastasized, and the number of lymph nodes removed at the time of surgery. The large number of positive lymph nodes with metastases also affects the prognosis.¹³

Based on this, the researcher wanted to know how the correlation between autophagy and glycolysis mechanisms with histopathological grading of penile SCC through the immunohistochemical expression of LC3B and GLUT-1.

2. Material and Methods

This research is analytic with a cross sectional approach, which was carried out at the Department of Anatomical Pathology, Faculty of Medicine, Universitas Sumatera Utara and the Pathology Unit of the H. Adam Malik Central General Hospital Medan, starting from January 2022 to May 2022.

The study samples were paraffin blocks and histopathological slides diagnosed as penile squamous cell carcinoma that had met the inclusion criteria and had been reviewed by 2 pathologists. The grading system used in this study is based on WHO criteria which are divided into grade I (well differentiated), grade II (moderately differentiated), and grade III (poorly differentiated).

The antibodies used were GeneTex LC3B Polyclonal 100 uL (1:100 diluted) and Bioenzy GLUT1 Polyclonal 100 ug (1:100 diluted). Immunohistochemical expressions of LC3B and GLUT 1 were categorized into positive and negative. LC3B expression was positive when stained brown in the cytoplasm of tumor cells, and GLUT-1 expression was positive when stained brown in the membrane and cytoplasm of tumor cells. The research data were analyzed using SPSS software with the Mann-Whitney test.

3. Results

The number of samples used in this study were 50 samples from paraffin block/slide histopathology diagnosed with penile SCC at the Anatomic Pathology Unit of H. Adam Malik Hospital Medan and the Department of Anatomical Pathology, Faculty of Medicine, Universitas Sumatera Utara. All samples have met the inclusion criteria.

Table 1 shows the frequency distribution of research subjects based on age. The highest age group was 41-50 years old with 15 people (30%) followed by subjects >60 years old with 14 people (28%). The least age group is 31-40 years old as many as 10 people (20%). The average age of patients with penile SCC in this study was 51.90 years with an age range of 31-83 years.

Table 1. Frequency Distribution of Research Subjects Based on Age

Age (years old)	Frequency	%
31 – 40	10	20
41 – 50	15	30
51 – 60	11	22
> 60	14	28
Total	50	100

Table 2 shows the frequency distribution of research subjects based on histopathological grading of penis SCC. The most histopathological grade was grade II with 22 samples (44%). The second highest grade is grade I with 17 samples (34%).

Table 2. Frequency Distribution of Research Subjects Based on Histopathological Grading of Penile SCC.

Grade	Frequency	%
I	17	34
II	22	44
III	11	22
Total	50	100

Table 3 shows the frequency distribution of research subjects based on the results of the examination of LC3B immunohistochemistry. Positive expressions were found in 46 samples (92%) while negative expressions were found in 4 samples (8%).

Table 3. Frequency Distribution of Research Subjects Based On The Results Of The Examination Of LC3B Immunohistochemistry

LC3B expression	Frequency	%
Positive	46	92
Negative	4	8
Total	50	100

Table 4 shows the frequency distribution of the research subjects based on the results of the examination of the immunohistochemical expression of GLUT-1 in the histopathological preparation of penile SCC. The results of the examination of GLUT-1 expression showed the same results as LC3B expression where positive expression was found in 46 samples (92%) while negative expression was found in 4 samples (8%).

Table 4. Frequency Distribution Of Research Subjects Based On The Results Of The Examination Of GLUT-1 Immunohistochemistry

GLUT-1 expression	Frequency	%
Positive	46	92
Negative	4	8
Total	50	100

Table 5 shows the results of the analysis of the correlation between the immunohistochemical expression of LC3B and the histopathological grading of penile SCC.

Table 5. Correlation Between The Immunohistochemical Expression Of LC3B and The Histopathological Grading Of Penile SCC.

LC3B expression	Grade			p*
	I	II	III	
Positive	15 (32,6)	22 (47,8)	9 (19,6)	0,818
Negative	2 (50)	0	2 (50)	

*Mann Whitney

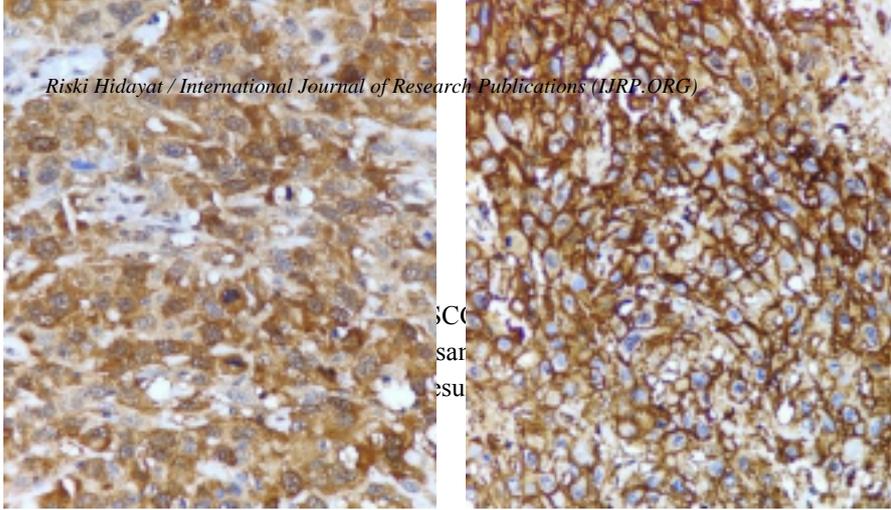
Of the 46 samples of penile SCC subjects with positive LC3B expression, there were 22 samples (47.8%) with grade II and 9 samples (19.6%) with grade III. Meanwhile, from 4 samples of subjects with negative expression results, there were 2 samples each with grade I and grade III results.

Table 4.6 shows the results of the analysis of the correlation between the immunohistochemical expression of GLUT-1 and the histopathological grading of penile SCC.

Table 6. Correlation Between The Immunohistochemical Expression of GLUT-1 and The Histopathological Grading of Penile SCC.

GLUT-1 expression	Grade			p
	I	II	III	
Positive	15 (32,6)	22 (47,8)	9 (19,6)	0,818
Negative	2 (50)	0	2 (50)	

*Mann Whitney



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B

Figure 1. A. positive expression of LC3B immunohistochemistry, B. positive expression of GLUT-1.

4. Discussion

In this study, the distribution of penile SCC based on age was found in the 41-50 years age group, with an average value of 50.90 years. These results are in line with the research conducted by Prayoga et al., in 2016 at the Sardjito Hospital Yogyakarta, where the most patients with penile SCC were in the age group above 40 years.¹⁴ Lestari et al., in 2021 conducted a study on the comparison of the incidence of penile cancer in 11 provinces in Indonesia in 2004-2013.² In this study, the highest age group for penile cancer patients in North Sumatra Province was in the 45-54 year age group. Dharma et al., in the same year also conducted a study on the prevalence of EGFR in penile cancer, where the age group of patients with penile cancer was mostly above 40 years (age range 41-60 years).¹⁵

In general, the age group of patients with penile SCC is found in the age group above 60 years. However, in this study, the highest age group found tended to be in the younger age group, namely 41-50 years. We think this may be related to risk factors for the habit of changing sexual partners that are often found today. Other factors that also influence are not doing circumcision and poor hygiene which is generally found in men in North Sumatra.

The frequency distribution of histopathological grading in this study showed that grade II was the most common histopathological grading, which was 22 samples (44%) and the second highest was grade I with 17 samples (34%). The results of this study are in line with research conducted by Zhang et al., in 2021 which identified lymph node metastases in penile cancer patients from 2004-2015. In this study, from 821 samples, 404 samples were found in grade II (49.2%), and the second highest was grade I with 260 samples (31.6%).¹⁶ This is different from the research conducted by Aita et al.,¹⁷ and Prayoga et al.,¹⁴ in 2016 which got the most histopathological grades from penile cancer patients was grade I and the second most was grade II. The results of this study are also not in accordance with the literature which says that grade I is the most common grade in

the incidence of penile SCC.¹⁸

We assume that although in this study grade II was more common, the difference between grade II and grade I was not too far off. So if we add a larger number of samples, we may get different results. Bias in the assessment of this grade may occur in samples with small tissue preparations, making grades difficult to assess.

In the frequency distribution of LC3B immunohistochemistry expression from 50 samples of penile SCC, 46 samples (92%) were positively expressed, and 4 samples (8%) were negative. The results of this study are in accordance with the literature which states that an increase in autophagy activity occurs in the process of developing a cancer. However, this increase in activity is still a debate whether the role of autophagy acts as an inhibitor or as a promoter of cancer.¹⁹ Positive LC3B expression was also found in normal cells. This is indeed commonly encountered due to autophagy activity which functions as homeostasis of a cell or tissue. However, positive LC3B expression in tumor cells was associated with microenvironment conditions in cancer cells, such as stress, nutrient deprivation, ROS, and tissue hypoxia that would trigger autophagy activity.

In the frequency distribution of GLUT-1 immunohistochemical expression of 50 penile SCC samples, the results were similar to those of LC3B expression, where 46 samples (92%) expressed positive and 4 samples (8%) expressed negative. The results of this study are in accordance with the literature which states that glycolytic activity increases in the process of developing a malignancy. The results of the same expression between LC3B and GLUT-1 also prove the involvement of increased autophagy activity which helps the glycolysis process in meeting energy needs in the growth of a cancer.⁸

The results of the analysis of the relationship between the immunohistochemical expression of LC3B and the histopathological grade of penile SCC in this study showed that the results were not statistically significant ($p = 0.818$), so there was no relationship between LC3B expression and the grade of penile SCC. This is in line with the study conducted by Zhu et al., in 2012 which analyzed the expression of Beclin-1 and LC3 in cervical SCC. The study concluded that there was no relationship between Beclin-1 and LC3 expression on age, tumor stage, pathological differentiation, and lymph node metastasis.²⁰ However, the results of this study differ from those obtained by Hu et al., in 2015 who investigated the expression of Beclin-1, LC3, and EGFR in cervical SCC, high grade cervical intraepithelial neoplasia (CIN), and normal cervical epithelium. The results of this study concluded that there was a correlation between the expression of Beclin-1, LC3, and EGFR to the three lesions. However, this study also concluded that there was no significant correlation between the expression of Beclin-1, LC3, and EGFR on the clinicopathological parameters of cervical SCC and the 5-year survival rate in cervical SCC patients.²¹ In addition, Chen et al., in 2013 also conducted the same study on SCC in the esophagus. From this study it was also concluded that the expression of LC3 and Beclin1 was not related to gender, age, tumor location, and response to treatment.²²

We argue that this insignificant result could be due to the unbalanced distribution of the number of samples between grades, so that if a sample is used that is evenly distributed between grades, it may get different results from this study. In addition, the researchers also argue that it is necessary to compare the expression of this LC3B against normal penile mucosa, benign lesions, and malignant lesions of the penis so as to strengthen the evidence for the association of this autophagy activity in tumorigenesis.

The results of the analysis of the correlation between GLUT-1 immunohistochemical expression and penile SCC grades in this study showed the same results as LC3B expression, where there was no relationship between GLUT-1 expression and penile SCC grade. This is not in line with the literature and several studies that have been conducted. Harshani et al., in 2014 analyzed

the expression of GLUT-1 in oral SCC, where the results of the study concluded that there was a significant correlation between GLUT-1 expression on clinical stage ($p=0.004$) and histopathological grade of oral SCC ($p=0,00001$).²³ Hidayat et al., in 2021 also conducted a study that analyzed the expression of GLUT-1 against benign, borderline, and malignant phyllodes tumors. This study found a significant correlation between GLUT-1 expression and phyllodes tumor grade.¹²

5. Conclusion

Based on the data and analysis that has been carried out, in this study it was concluded that there was no significant correlation between the histopathological grading of penile SCC with the immunohistochemical expression of LC3B and GLUT-1.

Competing interests

Author has no financial interests relevant to product or company described in this article.

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