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The Role Protein Sonic Hedgehog in Carcinoma Basal Cell

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A B S T R A C T

Introduction: Basal cell carcinoma (KSB) is a non-melanoma skin cancer (KKNM), which is most commonly found compared to other skin cancers. KSB originates from stem cells in the bulk of hair follicles or inter-follicular epidermis, through the Sonic hadgehog (SHH) activation pathway, an increase in *Sonic hadgehog* (SHH) protein expression, involving *Patches protein* (PTCH), *smothened protein* (SMO), in the form of increased protein transcription activation *Glia* (GLI) in the nucleus, binds to DNA to initiate tumor-aggressive growth and tissue. **Objective:** to determine the relationship between *Sonic hadgehog* (SHH) expression and non-aggressive and aggressive basal cell carcinoma. **Methods:** The study was carried out in an observational laboratory with 35 primary KSB patients, the tissue was taken using elliptic biopsy technique, made paraffin block specimens for histopathological examination of the subtype of KSB consisting of 20 non-aggressive KSB patients, namely nodular and superficial KSB; 15 patients with aggressive KSB were pigmented KSB; Infiltrates KSB, micronodular KSB, metypical KSB (basosquamous KSB) and SHH immunohistochemical (CPI) examination using SHH antibodies, in the Anatomy Pathology section, FK Unsri / RSMH Palembang. The characteristics of KSB patients were recorded, namely sex, age, occupation based on the length of exposure to BC, namely exposure <3 hours / day, exposure 3-6 hours / day, exposure ≥ 6 hours / day. The data were processed using the *Statistical Analysis Software Package* (SPSS) version 20.0 (IBM *Corporation*), tested with *Pearson correlation test and chi square test* and presented in the form of diagrams, and narrative tables. **Results**: *Pearson's* test showed a significant correlation between the clinical features of KSB and the histopathologic features of non-aggressive and aggressive types of KSB (p 0.020), there was a significant relationship between the subtypes of histopathologic features of KSB with non-aggressive and aggressive types of KSB (p 0.000), there was a significant relationship between strong SHH expression and BCC aggressive compared to non-aggressive KSB, p 0.000 (p <05α), and r = 732 **Conclusion**: There is a relationship between SHH expression and KSB aggressiveness. The increase in strong SHH expression shows the aggressiveness of KSB, SHH expression can be used as a biological gene target both as a prognostic indicator and can be used as a target for treatment of aggressive KSB, especially in the elderly.

1. Introduction

Basal cell carcinoma (KSB) or basalioma, basal cell epithelioma, rodent ulcer is a non-melanoma skin cancer with the characteristics of slow-growing, non-aggressive, locally invasive, although rarely aggressive, with far metastases ranging from 0.028% - 0.5% [1], especially attacking the elderly (Elderly), age range ≥ 60 years, more men than women, locations especially in sun-exposed areas (BC) such as the face, rarely attacking areas not exposed to SM[2], especially attacking Caucasians, rarely people Asia, in Taiwan the incidence of KSB is around 0.015% [3], research in Palembang at tertiary health facilities RSMH Palembang 2020 found 162 (46.8%) primary cases of KSB, male2: female 1: 1.13[4], there was an increase in

the incidence of KSB 3 times compared to previous studies 2015 as many as 35 (17.4%) primary cases of KSB

Until now, the etiology of KSB is still unclear. Several risk factors play a role in the incidence of KSB, especially the long and intermittent exogenous factor of sun exposure (SM), and endogenous factors, including genetic factors, including increased *sonic haghehog expression* (SHH) [2,5]

According to WHO, the histopathological picture of KSB based on the *growth pattern* consists of non-aggressive type of CSF, namely nodular and superficial subtypes; BCC aggressive types, namely infiltrates, micronodular, *morphea* type,[6–8] metypical type. Although it is still controversial, in the era of stem cell theory, a study of transgenic mouse models, it was proven that KSB originates from epidermal stem cells in the hair follicle bulge and interfollicular epidermis (IFE), so it is thought that KSB is a skin cancer derived from stem cells. Previous studies have shown that the pathogenesis of KSB is associated with deviations in the activation pathway of the Haghehog (HH)[9] signal mechanism, in the form of increased SHH expression, binds to PTCH as a tumor suppressor gene, causes *loss of function* PTCH, *Smoothened phosphorylation* (SMO) causes *gain of function* of SMO, translocates to the nucleus, binds to nuclear DNA, promotes the activation of glia protein transcription (GLI) for the initiation of growth and development,[10]. Kim's research (2019) shows that an increase in SHH expression occurs in the initiation phase of the KSB carcinogenesis process. [11]

The clinical picture of KSB, depicts very complex and varied and often non-specific, and has been going on for a long time, is commonly found in the elderly, generally it is found in advanced stage KSB, so it is difficult to know whether it is non-aggressive or aggressive, immunohistochemical examination (IHK) SHH is needed to determine the relationship between SHH with non-aggressive and aggressive KSB, so that SHH can be used as a marker to distinguish non-aggressive and aggressive KSB, perhaps it can be used as a therapeutic target in the treatment of aggressive KSB, especially in the elderly

2. Research Methods

This research was conducted in an observational laboratory with the research subjects as primary KSB patients in the Dermatology and Venereology section of the FK Unsri / RSMH Palembang, the period of January 2018-August 2018, as many as 35 patients were clinically non-aggressive and aggressive. paraffin was performed histopathologic examination to determine the subtypes of KSB consisting of 20 non-aggressive KSB patients, namely nodular KSB and superficial KSB; 15 patients with aggressive KSB were pigmented KSB; Infiltrates KSB, micronodular KSB, metypical KSB (basosquamous KSB) and continued with Sonic Hadgehog (SHH) immunohistochemical examination (IHK) in the Pathology Anatomy Department, Faculty of Medicine, Universitas Sriwijaya / RSUP Dr. M. Hoesin Palembang using SHH antibody to prove an increase in SHH levels in non-aggressive and aggressive KSB. The characteristics of KSB patients were recorded, namely sex, age, occupation based on the length of exposure to SM, namely exposure <3 hours / day, exposure to 3-6 hours / day, exposure ≥ 6 hours / day. Informed consent was carried out and had received approval from the Ethics Committee of FK Unsri / RSMH Palembang. The data were processed using the *Statistical Analysis Software Package (SPSS)* version 20.0 (IBM Corporation), tested with the *Pearson correlation test and chi square* test and presented in the form of diagrams, tables and narratives.

3. Result

Diagnostic laboratory observation study to

determine the relationship between SHH expression in non-aggressive and aggressive KSB with 35 specimens of KSB patients, with 20 specimens of non-aggressive KSB and 15 specimens of aggressive KSB. Statistic using *Pearson correlation test and chi square test.*

Characteristic relationship The histopathologic features of non-aggressive and aggressive KSB are shown in **Table 1**

From **Table 1,** based on age, there is no meaningful relationship between age and aggressive and non-aggressive KSB. (p = 0.489). Based on gender, 18 patients (51.4%) were found to be male compared to 17 patients (48.6%), there was no significant relationship between sex and non-aggressive and aggressive KSB (p 0.407). Based on the relationship between work and sunlight (SM), 11 patients (31.4%) worked <3 hours / day, 10 patients (28.6%) worked > 3 hours - 6 hours / day (28.6%) and worked ≥6 hours / day. as many as 14 patients (40.9%) 0, there was no significant relationship between the length of work of the non-aggressive and aggressive KSB patients with the length of SM exposure (p = 0.318).

In this study, the relationship between clinical features with non-aggressive and aggressive KSB. The clinical picture consists of nodular type, nodular-pigmented type; nodular-ulcerative type, while the histopathologic features consist of: non-aggressive KSB (nodular type, superficial type KSB) and aggressive KSB (infiltrates / morphea type KSB, micronodular type KSB, metypical type KSB) and can be seen in **Table 2**

In **Table 2,** the relationship between clinical features and non-aggressive and aggressive KSB. Histopathologically non-aggressive CSB was 20 patients (71.38%) which was in accordance with the clinical features of the nodular type KSB consecutively as many as 15 patients (57.1%). nodular pigmented as many as 3 patients (8.57%), nodular ulcerative type were 2 patients (5.71%), while histopathologically aggressive KSB were 15 patients (42.9%) which according to the clinical picture consisted of consecutive types of KSB. nodular pigmented as many as 10 patients (28.6%), nodular ulcerative type as many as 5 patients (14.3%), Pearson chi square test, there was a significant relationship between the clinical features of KSB with the histopathologic features of non-aggressive and aggressive types of KSB (p 0.020)

In **Table 3**, histopathologically, the relationship between subtypes of KSB with non-aggressive and aggressive KSB. In a row, there were 20 patients (57.13%) consisting of 13 patients (37.14%) of the nodular type, 3 patients (8.57%) of the superficial type, and 4 patients (11.42%), aggressive KSB as many as 15 patients (42.9%) consecutively consisting of 7 patients (20%) micronodular type, 6 patients (17.14%) infiltrates type KSB, and 2 basosquamous type KSB patients (5.71%). Pearson *chi-square* test, showed that there was a significant relationship between the subtypes of the histopathological features of CSB with non-aggressive and aggressive types of KSB (p 0.000).

In **Table 4,** the relationship between SHH expression and non-aggressive and aggressive BCC shows that a total of 14 patients (40%) with strong SHH expression (3+) were found to be aggressive in 12 patients (34.29%). patients (5.71%). From 15 patients (42.9%) with moderate SHH expression (+2), 14 patients (40%) had non-aggressive KSB; Aggressive KSB in 1 patient (2.86%), while weak SHH expression (1+) was found in 6 patients (17.14%), there were 4 patients (11.43%) of non-aggressive BCC and 2 patients (11.43%). 17.14%). Pearson test showed that there was a significant relationship between strong SHH expression and aggressive KSB compared to non-aggressive BCC, p 0.000 (p <05α), r = 732; showed that there was a relationship 7 times higher in the increase of strong SHH expression compared to moderate and weak SHH expression on the aggressiveness of KSB. The conclusion is that there is a significant relationship between strong SHH expression and histopathological features of KSB, there is a tendency for development and

growth to become aggressive KSB (p 0.000; r 0.732)

Table 1. Correlation of the characteristics of KSB patients with non-aggressive and aggressive KSB

Variable	Basal Cell Carcinoma (KSB)			P value
	Non-aggressive KSB	Aggressive KSB	Total	
	N (%)	N (%)	N (%)	
Age				
- 33 – 42 years old	1 (2.86%)	3 (8.57%)	4 (11.43%)	
- 43 – 52 years old	54(11.43%)	4 (11.43%)	8 (22.86%)	
- 53 – 62 years old	65 (14.29%)	3 (8.57%)	8 (22.86%)	0.489
- 63 – 72 years old	3 (8.57%)	4(11.43%)	7 (20%)	
- 73 – 82 years old	4 (11.43%)	1(2.86%)	5 (14.29%)	
- 83 – 92 years old	1 (2.86%)	0 (0%)	1 (2.86%)	
Gender				
- Male	12 (34.29%)	6 (17.14%) 18		0.407
- Female	8 (22.86%)	9 (25.71%) 17		
Profession				
- SM exposure ≥6 hours / day	8 (22.86%)	3 (8.57%)	11	
- Intermittent exposure to SM 3 - 6 hours / day	6 (17.14%)	4 (11.34%)	10 (28.6%)8	0.318
- SM exposure ≤3 hours / day	5 (14.29%)	8 (22.86%)	13 (37.14%)	

Table 2. The relationship between clinical features and non-aggressive and aggressive KSB

Clinical Features	Histopathologic diagnosis				P value
	Non-aggressive KSB		Aggressive KSB		
	N (%)	N (%)	N (%)	N (%)	
Nodular type	15	57.1	-	-	
Pigmented nodular type	3	8.57	10	28.6	0.020
Nodular-ulcerative type	2	5.71	5	14.3	
Total	20	71.38	15	42.9	

Table 3. The relationship between the histopathological features of the sub-type of KSB with non-aggressive and aggressive KSB

Histopathologic Features	Non-aggressive KSB		Aggressive KSB		Total		P value
	n	%	n	%	n	%	
Nodular KSB	13	37.14	-	-	13	37.14	
Superficial KSB	3	8.57	-	-	3	8.57	
Pigmented KSB	4	11.43	-	-	4	11.43	
Micronodular KSB	-	-	7	20	7	20	0.000
KSB infiltrates	-	-	6	17.14	6	17.14	
Basosquamos / metypical KSB	-	-	2	5,71	2	5.71	
Total	20	57.14	15	42.9	35	100	

4. Discussion

Epidemiological research shows that KSB affects more white people than Asians. Recently in Palembang there has been an increase in the frequency of KSB, Odel et al's research (2020) has proven that 1 (3.9%) of the primary KSB affects especially the elderly in the range of 60 years and over, mostly affects women: men by 1.13 : 1[4]. Yap's previous research on Asians in Malaysia showed that KSB attacks both men and women equally, the ratio is 1.05; especially attacking the old age range of 60.9 years.[12]

In the study (table 1), KSB was found in men as many as 18 (51.42%), women 17 (48.57%,) with a ratio of 1.05: 1, the most age consecutively 53 - 62 years as many as 9 patients (22.86%), and ages 43-52 were 9 patients (22.86%), aged 62-72 were 7 patients (20%). There was no statistically significant difference between sex, age and the incidence of KSB (p≥0.05). In this study, 9 patients

(22.86%) were found aggressive KSB aged 43-52 years, and 9 patients aged 53-62 years (22.86%). Previous research showed that aggressive KSB was more prevalent in age. younger than the elderly, generally the prognostic is poor.13 Further research is needed to determine the relationship between young age and KSB aggressiveness. KSB is benign compared to non-melanoma skin cancer, but often causes local destruction, which affects quality of life, although rarely becomes aggressive, can metastasize far in the 0.028-0.5% range, causes high morbidity, often difficult to determine further treatment.[14]

In the study (**Table 2**) there was a relationship between clinical and histopathological features. In non-aggressive type KSB was found as many as 20 patients (71.38%), consecutively consisting of the nodular type as many as 15 patients (57.1%), nodular pigmented as many as 3 patients (8.57%), nodular ulcerative type as many as 2 patients (5,71%). In the aggressive type KSB found 15 patients (42.9%) consecutively consisting of 10 patients (28.6%) nodular pigmented type and 5 patients (14.3%) nodular ulcerative type. *Chi square* test showed that there was a significant relationship between the clinical features of KSB and the histopathologic features of the non-aggressive and aggressive types (p 0.020). According to the literature, there is a significant relationship between the clinical features of nodular type KSB and non-aggressive KSB. In general, Asian people have Fitzpatrick skin types 3 and 4, clinically KSB is often found with nodular pigmented and ulcerative types, clinically it can show non-aggressive and aggressive types of KSB, especially in the elderly, often neglecting treatment.15 This study finds more features. clinical KSB pigmented nodular type and nodular ulcerative type KSB, including aggressive KSB. It shows that the clinical picture of KSB is very complex and varied, so it is necessary to know the biological molecular markers of SHH as a marker for aggressive KSB.

In the study (**table 3**) there was a relationship between the histopathologic features of KSB subtypes with non-aggressive and aggressive KSB, non-aggressive KSB consisting of 13 (37.14%) nodular-type KSB, 4 patients (8,57). %), Pigmented KSB were 4 patients (11.42%). Aggressive KSB consisted of 7 micronodular patients (20%), 6 patients (17.14%) infiltrates subtypes, 2 metypical subtypes (5.71%).

Until now there has been no agreement with the researchers. Most of the researchers divided the CLS classification based on *growth patterns,* which had a significant relationship between non-aggressive and aggressive types of KSB with molecular biological activity of SHH. Previous research on sporadic KSB showed an increase in SHH expression, it was proved that the increase in SHH played a major role, in the development and aggressiveness of KSB[16], Fecher in his study proved that the treatment of KSB by inhibiting the signaling *pathway* of the SHH target in advanced stage KSB reduces severe damage and reduces morbidity of action. surgeri1[7]

Basal cell carcinoma (KSB) is the most common skin cancer with a very complex and varied clinical picture, possibly influenced by extrinsic factors and intrinsic factors[18,19]. The influence of environmental factors as extrinsic factors, especially prolonged and intermittent exposure to sunlight (SM), both Ultraviolet A (UVA) and B (UVB) rays affect the growth and aggressiveness of KSB[20]. In this study, statistically, there was no effect of SM exposure on the incidence of KSB (p≥ 0.05). Perhaps the aggressiveness of KSB is influenced by the collaboration between extrinsic factors, especially SM with intrinsic factors in the form of genetic influences, namely an increase in protein expression of the SHH gene. Further research is needed, involving several DV education centers in Indonesia to determine the relationship between SM and increased SHH expression and KSB aggressiveness.

Although it is not clear the role of the *sonic hadgehog* (SHH) gene in the growth and aggressiveness of KSB. Research in the mouse

model proves that the carcinogenesis process in the skin, breast, brain, prostate, gastrointestinal tract and pancreas, shows that there is an increase in SHH expression, there is *a gain of function* in PTCH expression as a tumor suppressor gene, increasing SMO protein expression, Glia transcription (GLI) as an oncogen. In the nucleus, increased activation leads to the initiation and aggressiveness of KSB21. Another study proved that in a transgenic mouse model, KSB is a cancer of the epidermal stem cells which is activated through a distortion of the HH pathway signal, in the form of an increase in SHH22–24 expression. Kim's research in Korea (2019) shows the involvement of SHH in the development of KSB, GLI1 can be used as a marker to differentiate KSB from other malignant skin tumors, although further research is needed.[11]

In the study (table 4) showed strong SHH expression (3+) as many as 14 patients (40%) consisted of 2 patients (5.71%) non-aggressive KSB and 12 patients (34.29%) aggressive KSB, while the SHH expression (2+) as many as 15 patients (42.86%) consisting of 14 patients (40%) non-aggressive KSB and 1 patient (2.86%) aggressive KSB p 0.000; r 0.732. In conclusion, there is a significant relationship between increased SHH protein expression and KSB aggressiveness.

Baskhi (2017) explains that in an extensive study, the presence of HH signal deviation plays a major role in sporadic KSB, *crosstalk* with target gene molecules increases GLI activity as an oncogen, causing increased tumor growth and metastasis. there is a decrease in the expression of the target gene neogenin 1 (NEO1) resulting in downregulation of SHH which is associated with the aggressiveness of KSB, although further research is needed25. Further research is needed with more samples regarding the relationship between SHH expression and the role of NEO1 with aggressive KSB

5. Conclusion

There is a relationship between increased SHH expression and aggressive KSB. Increased expression of SHH can be used as a target to determine the aggressiveness of KSB, and as a prognostic indicator and is used as a target for treatment of aggressive KSB, especially the elderly.

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