EXPRESSIONS OF STEM CELL FACTOR AND TYROSINASE POSITIVELY CORRELATE WITH EXPRESSION OF Ki-67 IN SEBORRHEIC KERATOSES

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ABSTRACT

Cellular proliferation of KS will increase secretion of derivate keratinocyte factors that is stem cell factor (SCF) which is a melanogenic cytokines that causes proliferation and differentiation of cells and increase the transcription of tyrosinase enzyme (TYR). It is not clear whether SCF and TYR play a role in cellular proliferation of SK. This study aims to determine the relationship between expressions of SCF and TYR with expression of Ki-67 as a proliferative marker in seborrheic keratosis.

This study used a cross sectional design with a sample of 67 SK lesions from patients who visited the Clinic of Dermato-Venerology Sanglah hospital, Denpasar. Statistical analysis in this study used linear regression test to analyze the relationship between the independent variables (expression of SCF and TYR) and the dependent variable (Ki-67).

Stem Cell Factor expression was found to be positively correlated with the expression of Ki-67 ($r=0.61, p<0.001$) with regression coefficient 0.61 ($t=6.22, p<0.001$) and constant -49. Correspondingly, the result indicated positive correlation between tyrosinase expression and Ki-67 ($r=0.61, p<0.001$) with regression coefficient 0.755 ($t=8.86, p<0.001$) and constant -6.02.

This study showed a strongly and positive correlation between the expressions of SCF, TYR with expression of Ki-67 which is a marker of cell proliferation.

Keywords: seborrhoeic keratosis, Ki-67, SCF, tyrosinase

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INTRODUCTION

Seborrheic keratosis (SK) is a benign tumor composed of keratinocytes which is accompanied by an increase pigmentation.¹ The tumor grows from epidermal cell and melanocyte proliferation.

Cell proliferation is induced by melanogenic cytokine secretion and growth factors secreted by keratinocytes, melanocytes and fibroblasts. Melanocytes with keratinocytes and fibroblasts interact through networks of paracrine, autocrine melanogenic cytokines.²,³ Relationship between melanogenic cytokine of SCF, tyrosinase enzyme with the cell proliferation mechanisms in SK are not yet fully understood.

The Ki - 67 antigen is one of proteins that regulates the cell cycle and can be evaluated using the immunohistochemical method. Ki-67 antibody is a biomarker of cell proliferation reacting nuclei with non - histone proteins are expressed at low during the G1 phase and early S phase, progressively and reached a maximum increased during the phases of mitosis, but it is not expressed in the phase G0.⁴

Stem cell factor protein is secreted by several cell types (keratinocytes, fibroblasts and endothelial cells), suggesting SCF plays an important role in physiological and pathological processes in the skin.⁵ Stem cell factor plays have a role in regulating epidermal melanogenesis.⁶

Tyrosinase is the main enzyme in melanogenesis. This enzyme plays a role performance synthesis of melanin that gives color in skin.⁷

This study aims to determine the relationship between expressions of SCF and TYR with expression of Ki-67 in seborrheic keratosis.

MATERIAL AND METHOD

Data and human skin sample

This study used cross sectional design. Human skin samples were obtained from skin biopsies of 67 tissues with SK, taken by minor surgery with a 4 mm punch biopsy, who had previously signed an informed consent and examination using FotoFinder System GmbH mobile dermoscopy to eliminate the other pigmented skin disease. Seborrheic keratoses lesions were taken from the areas of face, neck and chest.
**Immunohistochemical staining**

Immunohistochemical staining of Ki-67, SCF and TYR were performed to all tissues. Tissue fixed in 10% buffered formalin, paraffin tissue blocks were cut at 3 micrometer thick. Pieces of tissue were placed on a poly L-lysine coated glass object. Deparaffinization was done with xylene, rehydrated with ethanol and blocking reaction with H₂O₂ and performed with antigen retrieval. Tissue incubated with primary antibody Ki-67 used rabbit anti-human polyclonal antibodies from Santa Crus Biotechnology with dilution of 1:100, incubated for 2 hours at room temperature. Stem cell factor staining used mouse anti-human monoclonal antibody from Santa Crus Biotechnology as primary antibody with dilution 1:100. Tyrosinase staining used mouse anti-human monoclonal antibody from Novus Biological as primary antibody with dilution 1:200. To show the binding sites of primary antibody, DAB staining and incubation were performed for 5 minutes. The last step was counterstaining by hematoxylin, dehydrated, cleared and calculation.

**Staining assessment**

Slides were examined using Olympus BX51 microscope with Image Optic Lab View and Image Raste software at 400 x optical magnification. The Ki-67 expression and SFF, TYR were calculated by percentage. The percentage was determined by numbers of Ki-67 positive cells divided the total number of cells in the basal layer. The calculation of percentage of SCF, TYR was similarly done.

**Statistical analysis**

Data were analysis in statistical SPSS program for window using univariate analysis to express the frequency distribution of characteristics of skin samples and using bivariate analysis (linear regression) to test correlation between SCF and TYR with Ki-67.

**RESULTS**

Subject characteristics (number, lesion diameter, color, type of lesion) were shown by Table 1.
Table 1. Characteristics of skin samples

<table>
<thead>
<tr>
<th>Sample Characteristics</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of lesion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-10</td>
<td>2</td>
<td>2.9</td>
</tr>
<tr>
<td>11-19</td>
<td>28</td>
<td>40.6</td>
</tr>
<tr>
<td>≥ 20</td>
<td>39</td>
<td>56.5</td>
</tr>
<tr>
<td>Type of lesion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Verruciform plaques</td>
<td>32</td>
<td>46.4</td>
</tr>
<tr>
<td>Domed shaped papule</td>
<td>25</td>
<td>36.4</td>
</tr>
<tr>
<td>Patch/macula</td>
<td>12</td>
<td>17.4</td>
</tr>
<tr>
<td>Diameter of lesion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 mm</td>
<td>5</td>
<td>7.2</td>
</tr>
<tr>
<td>3 mm</td>
<td>19</td>
<td>2.75</td>
</tr>
<tr>
<td>4 mm</td>
<td>29</td>
<td>42.0</td>
</tr>
<tr>
<td>&gt;4mm</td>
<td>16</td>
<td>23.2</td>
</tr>
<tr>
<td>Color of lesion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dark brown</td>
<td>35</td>
<td>50.7</td>
</tr>
<tr>
<td>Light brown</td>
<td>34</td>
<td>49.3</td>
</tr>
</tbody>
</table>

In Table 1 it was shown that the highest proportion of number lesions ≥ 20 (56.5%), verruciform plaques type (46.4%), diameter of lesion 4 mm (42.0%) and the color of dark brown lesions was 50.7%, the most commonly found.

Table 2. Correlation between Ki-67 and SCF, and TYR

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Coefficient (r)</th>
<th>Degree of freedom (df)</th>
<th>p</th>
<th>R square (R^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCF (%)</td>
<td>0.61</td>
<td>1</td>
<td>&lt;0.001</td>
<td>0.367</td>
</tr>
<tr>
<td>TYR (%)</td>
<td>0.74</td>
<td>1</td>
<td>&lt;0.001</td>
<td>0.540</td>
</tr>
</tbody>
</table>
Table 2 showed Pearson’s correlation analysis that correlated SCF and TYR with Ki-67. Both of independent variables (SCF and TYR) were found to be significantly associated with Ki-67, furthermore, the linear model developed from both predictors were significant at p<0.05. The correlation coefficient for SCF is 0,61 (df=1, p<0,001), meaning SCF was strongly and linearly correlated with Ki-67. This correlation had R² of 0.36,7 meaning that the proportion of variance in ki-67 variable which can be explained by the SCF variable by 36.7%. Meanwhile, the coefficient for TYR is 0,74 (df=1, p<0,001), meaning TYR was also strongly and linearly correlated with Ki-67. The Pearson’s correlation test between TYR and ki-67 resulted in stronger R² that is 0.54, meaning the proportion of variance in the Ki-67 variable which can be explained by the TYR variable is 54%.

![Scatter plot of correlation between expression of SCF with Ki-67.](image)

Figure 1. Scatter plot of correlation between expression of SCF with Ki-67.

Figure 1 showed scatter plot of correlation between expression of SCF with Ki-67, which showed regression equation between SCF and Ki-67 with regression coefficient 0.61 (t=6.22, p<0.001) and constant -49. It mean that every unit increase in SCF, a 0,61 unit increase in Ki-67 is predicted, holding all other variable constant.
Figure 2. Scatter plot of correlation between expression of TYR and Ki-6.

Figure 2, also showed scatter plot of correlation between expression of TYR and Ki-67, which showed regression equation between TYR and Ki-67 with regression coefficient 0.755 (t=8.86, p<0.001) and constant -6.02. It mean that every unit increase in TYR, a 0.755 unit increase in Ki-67 is predicted, holding all other variables constant.

DISCUSSION

The study reported that the proportion of the highest number of lesions ≥ 20 (56.5%), the type of the lesion verruciform plaques at the most, diameter of lesions 4 mm (42.0%) and the color of dark brown lesions (50.7%) at the most found. Research Yeatman et al reported the diameter of the lesions on sun-exposed areas < 3 mm were (16%) and diameter of lesions > 3mm were 41%, type of lesions verrucose plaque were 23% and patches type were 38% in exposed areas and dark brown lesions were 33% and light brown color were 31% in areas exposed to sunlight. Exposure to sunlight is an indirect risk factor of SK growth and development. The skin is the outer organ that is most frequent and prolonged to exposure, resulting in changes of pigmentation and skin cell hyperproliferation because of a decreased ability to repair damaged cells and the change of gene expression that is associated with the regulation of cell growth.

The correlation between SCF and TYR with Ki-67 in this study were found to be significantly strong, high expression of SCF and TYR were positively associated with high expression of Ki-67. Stem cell factor binds to the receptor c-kit, in turn causes
phosphorilation variety of substrates and signaling molecules associated with various signaling pathways which in turn activates the Ras-MAPK signaling pathway in melanosit.\textsuperscript{10} Stem Cell factor is important role in migration, cell survival of melanocytes in the embryonic phase and formation melanin in adulthood. Activation of signaling of the MAPK (Mitogen Activated Protein Kinase) pathway causes phosphorylate MITF is a transcription factor, through the ERK (extracellular signal-regulated kinase) phosphorylation, thereby increasing the transcription of enzymes that play a role in the process of melanogenesis is tyrosinase, tyrosinase-related protein 1 and tyrosinase-related protein 2. Activation of this process will increase the synthesis of melanin, proliferation, differentiation and formation of dendrites on melanosit.\textsuperscript{11,12} Other studies also support, that intracutaneous injection of SCF are increasing the numbers, sizes, and dendrites of melanocytes in skin.\textsuperscript{6} It can be concluded, there is a relationship between melanocyte cell proliferation by SCF melanogenic cytokine and enzyme tyrosinase.

**CONCLUSION**

It is concluded that the expressions of SCF and TYR have a strong correlation with Ki-67 which is marker of proliferation cell in in seborrheic keratosis.

**REFERENCES**


