ABSTRACT

of the PhD thesis entitled

The immunomorphometric study of skin malignant tumors

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Scientific coordinator: Prof. Dr. Egyed-Zsigmond Emeric

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Introduction

During the last decades we have assisted to the multiplication of the cases of skin malignant tumors and basal cell carcinoma with benign evolution with little or no chance of metastasis, as well as of malignant tumors with fast proliferation and unfavorable prognosis.

Aim of study

The proposed study aims to establish the aggressiveness of these skin malignant tumors by the usage of some immunohistochemical markers specific for the tumor angiogenesis, cell adhesion and stromal components and by the determination of some correlations between the intensity of immunohistochemical reactions and the histopathological malignancy degrees.

Material and method

This study has been performed during a period of five years and included skin malignant tumor biopsies processed within the Department of Pathological Anatomy of the Emergency Clinical Hospital of Mureş county. All cases have been revised on basis of the typical colorings; I have chosen representative cases for the immunohistochemical processing and then for the morphometric analysis. The antibodies used for the study of tumor angiogenesis are the following: CD31, CD105,

VEGF and FLK1, the Anti-E-cadherin antibodies for the study of cell adhesion, and the SMA and GFAP antibodies for the emphasis of myofibroblasts.

These have been submitted to the examinations of digital morphometry; using the ImageJ program, I have determined a relation for each digital image, among the brown and blue (positive/negative) territories, indicating the immunopositive surface (SI). I have obtained SI values in 5 microscopic fields of the same section of which I have calculated the average percentage of the immunopositive surfaces – PMSI.

As statistical methods I used the T test for independent variables (continuous variables with normal distribution in Kolmogorov-Smirnov test) and the ANOVA test in order to compare the average differences of more than two samples. The "optimal binning" procedure, by statistical data processing reveals a table with descriptive statistics, a model of entropy and a final table with the established limits in which the malignancy degrees are enclosed numerically and as a percentage.

Results

The examined group included 480 cases of skin malignant tumors during a period of 5 years 2003 – 2007, containing basal cell carcinoma, spinal cell carcinoma and malignant melanoma.

In basal cell carcinoma I have identified a statistically significant difference (p-0,05) between the PMSI-CD31 value of 3,28 in the first degree and of 1,87 in the 2^{nd} and 3^{rd} degrees. A more obvious difference (p-0,0001) is noticed between the PMSI-CD105 values in the 1^{st} degree in comparison with the average CD105 values in the 2^{nd} and 3^{rd} degrees. In case of PMSI of VGEF I have not identified statistically significant differences (p-0,32). By the application of Student test I have identified a statistically significant difference (p-0,0001) between the average FLK1 values in the 1^{st} degree in comparison with the FLK1 values in the 2^{nd} and 3^{rd} degrees. The model of entropy shows that among the used immunohistochemical reactions, FLK1 has the highest accuracy with the value of 0,232 and the lowest accuracy is presented by CD105 with the value of 0,360. The study of cell adhesion shows by the application of the Student statistical test a p less than 0,0001, meaning statistically significant differences of this

immunohistochemical reaction to the three degrees of malignancy. By the application of the statistical Student test, the study of myofibroblasts shows statistically significant differences between the SMA values as immunohistochemical reaction to the three degrees of malignancy (p-0,0001), in exchange, there have not been noticed statistically significant differences in case of GFAP (p-0,11). The model of entropy shows that the highest accuracy is represented by SMA with the value of 0,094 and the lowest accuracy is represented by GFAP with the value of 0,471. The values higher than 0,76 show lower degrees of malignancy and the values of 0,76 show higher degrees of malignancy.

In spinal cell carcinoma the Anova test emphasizes that the p value id lower than 0,001, so there are statistically significant differences of the used immunohistochemical reactions. The model of entropy shows that among the used immunohistochemical reactions the highest accuracy from the angiogenesis point of view is represented by CD31 with a value of 0,767 and FLK1 has the lowest accuracy with a value of 1,611. PMSI emphasizes the values up to 9,54 of CD105 which show the 1^{st} malignancy degree and the values between 9,54 – 18,07 show the 2^{nd} and 3^{rd} malignancy degrees while the values over 18,07 show the 3^{rd} and 4^{th} degrees of spinal cell carcinoma. These barrier values achieve a maximum discrimination among the 4 degrees of malignancy. We may find similar correlations for CD31, for VEGF and for FLK1. In the study of cell adhesion, by the application of Anova test I have identified a statistically significant difference between the averages of the immunohistochemical values in E-cad according to the four stages (p-0,0001). The determination of some optimal barrier values (cut-point or cut-off point) of PMSI and SMA for the degrees of malignancy in spinal carcinoma emphasizes the values up to 2,34 of SMA showing the 1^{st} and 2^{nd} degrees of malignancy, the values among 2,34 – 5,25 showing the 2^{nd} and 3^{rd} degrees of malignancy and the values over 5,25 showing the 3^{rd} and 4^{th} degrees of spinal cell carcinoma. Applying the Anova test I have identified statistically significant differences between the average values of SMA (p-0,0001) and GFAP (P-0,01).

In malignant melanoma, by the application of Anova test between the PMSI values, I have identified statistically significant differences according to the malignancy stages, in

case of CD31 (P-0,0001), CD105 (p-0,0001), VGEF (p-0,0001) and in case of FLK1 (p-0,01). The model of entropy reveals the fact that the highest accuracy from the point of view of the angiogenesis of malignant melanoma is represented by VEGF with a value of 0,102 and the lowest accuracy is represented by CD31 with a value of 0,825. The Anova test applied shows a statistically significant difference between the averages of immunohistochemical average values of E-cad according to the malignancy stages (p-0,0001). The cut-off point determination shows that the PMSI values higher than 2,94 of E-Cadherins show lower degrees of malignancy and the values lower than 2,94 show higher degrees of malignancy. The Anova test applied reveals a statistically significant difference between the PMSI values in case of SMA according to the malignancy stages (p-0,0001), in exchange I have not identified a statistically significant difference between the average values of GFAP (p-0,25). The PMSI values higher than 6,20 in SMA show higher degrees of malignancy, and the values lower than 6,20 show lower degrees of malignancy.

Conclusions

Once with the increase of the malignancy degree in all three categories of tumors, we notice the increase of CD105, VEGF and FLK1 expressions, excepting CD31 whose expression decreases toward the higher degrees of malignancy in basal cell carcinoma and increases toward the higher degrees of malignancy in spinal cell carcinoma and malignant melanoma. The study of cell adhesion emphasizes the decrease of E-cadherin positivity once with the increase of the malignancy degree in basal cell carcinoma and in spinal cell carcinoma as well as in malignant melanoma. In basal cell and spinal cell carcinoma the appearance of SMA and GFAP immunohistochemical markers in stroma indicates the increase of tumor aggressiveness and higher degrees of malignancy, in contrast with malignant melanoma where these markers are present both in stroma and in parenchyma, no matter the tumor stage.

Key words

Skin malignant tumors, angiogenesis, cell adhesion, myofibroblasts