 DOI: 10.33892/aph.2021.91.332-333

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Alpha-1-Acid Glycoprotein as a Diagnostic Biomarker for Malignant Melanoma

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Keywords: *Alpha-1-acid glycoprotein, biomarker, glycosylation, linear discriminant analysis, malignant melanoma*

1. Introduction

Malignant melanoma is a skin tumor that arises from melanocytes. Although it represents less than 5% of all cutaneous malignancies, its high mortality rate and severe metastatic potential establish the need to develop specific and sensitive methods to recognize the disease and provide less invasive alternatives to traditional diagnostic procedures including histopathology and immunohistochemistry. To date, the available serological biomarkers, such as lactate dehydrogenase and S100B protein have been of prognostic value only. They show a better correlation with advanced clinical stages as well as the presence of metastases and have limited diagnostic usefulness. Among several natural glycoconjugates, alpha-1-acid glycoprotein (AGP), is one of the characteristic, highly glycosylated and frequently studied protein fraction of human serum, with a molecular mass of 41–43 kDa. Irregular glycosylation of the molecule was described in several cancer types, highlighting the relevance of AGP as a promising biomarker for malignant diseases [1]. Given that the diagnostics of malignant melanoma is deficient in proper bio/tumor markers, the present work aimed to conduct a comprehensive clinical study focusing on the alterations recognized in the glycosylation pattern of AGP in melanoma.

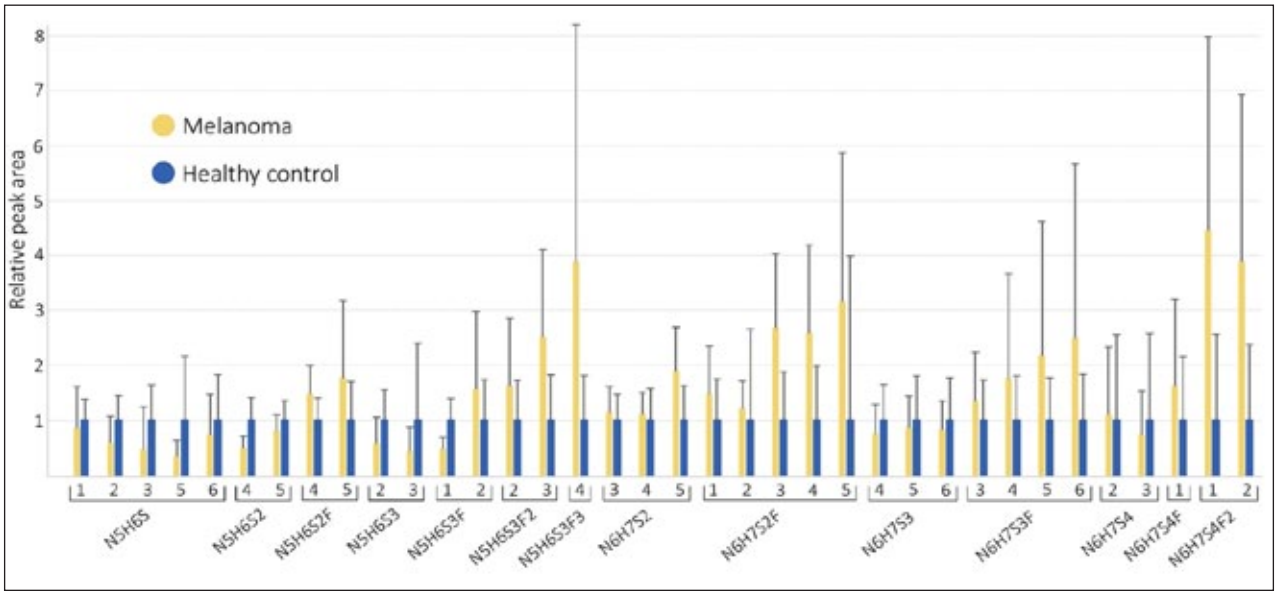

Figure 1 *Averaged relative peak areas of the isomers demonstrating characteristic changes between the groups Values of the melanoma group were normalized to the control group. N refers to N-acetylglucosamine, H to hexoses (galactose and mannose), S to sialic acid and F to fucose units*

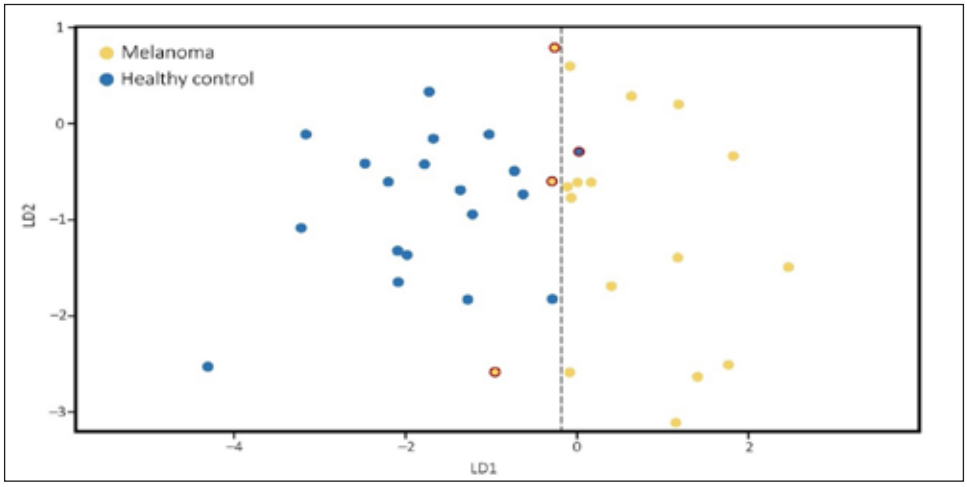

Figure 2 *Classification of the 37 samples using LDA Incorrect classifications are marked with red outlines*

2. Methods

Serum AGP of 18 high-risk melanoma patients and 19 healthy individuals were isolated. The carbohydrate content of the samples was released enzymatically and derivatized with anthranilic acid. Derivatives were subsequently analyzed by hydrophilic interaction liquid chromatography tandem mass spectrometry, and the result were evaluated by linear discriminant analysis (LDA). The study was approved by the Scientific and Research Ethics Committee of the Medical Research Council, Budapest, Hungary (SE RKEB 30/2020).

3. Results

To compare samples from affected and control individuals, relative peak area of each isomer was expressed as the percentage of the sum of the peak areas in a given sample. To further reduce the complexity of data and enhance visual comparison, relative peak areas of glycan were averaged within the two classes (malignant and control), then resulting values of the cancerous sample group were normalized to the control group. As **Figure 1** illustrates, overexpression of fucosylated glycans was recognized in the melanoma group, especially in more branched chains. An increase in some triantennary (N5H6S3F, N5H6S3F2, N5H6S3F3) and tetraantennary oligosaccharides (N6H7S4F, N6H7S4F2) also revealed that the extent of overexpression is seemingly proportional to the number of fucose units connected to the sugar backbone. This indicates directly the crucial relevance of fucosylation in the malignant process. Downregulation in the nonfucosylated counterparts (N5H6S2, N5H6S3, N6H7S3) of some glycans observed may be a consequence of increased fucosylation in melanoma samples.

When isomers of the same glycan were compared directly, an increased ratio of the isomers containing more α-2,6-linked sialic acids in the disease samples was observed in many cases. This was most pronounced in the ratio of isomers 4 and 5 of N5H6S2, 1, 2 of N5H6S3F, 3, 5 and 4, 5 of N6H7S2, as well as isomers 2 and 3 of N6H7S2F.

Linear discriminant analysis (LDA) combining 10 variables with the highest discriminatory power was employed to categorize the samples based on their glycosylation pattern (**Figure 2**). Performance of the method was tested by cross-validation, resulting in an overall classification success rate of 96.7%, significantly outperforming S100B protein (64.9%) in the population studied.

4. Conclusions

AGP was shown to be markedly superior to routine serological marker, S100B protein the population studied as regards the sensitivity and negative predictive power. Therefore, the results presented here indicate that the changes recognized in the sugar composition of AGP at the glycan structural level can serve as a powerful biomarker in the diagnosis of malignant melanoma.

5. Acknowledgements

The work was financed by the Higher Education Institutional Excellence Programme of the Ministry of Human Capacities in Hungary, within the framework of the molecular biology thematic programme of Semmelweis University.

References

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