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Melanogenesis Affects Overall and Disease-Free Survival in Patients with Stage III and IV Melanoma

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Abstract

Because melanogenesis can affect immune responses to and chemotherapy and radiotherapy for melanoma, we analyzed overall survival (OS) and disease-free survival (DFS) times in melanoma patients in relation to the degree of tumor pigmentation. Clinicopathologic data were obtained from the Oncology Centre, Prof. Franciszek Łukaszczyk Memorial Hospital, Bydgoszcz, Poland. The OS and DFS analyses were performed using the log-rank test, whereas differences between mean/median OS and DFS (days) were analyzed using the Student *t* test. In patients with metastatic disease, those with melanotic melanomas exhibited significantly shorter DFS and OS than those with amelanotic lesions. Similarly, melanin-producing lymph node metastases were linked to shorter OS and DFS, which was confirmed by a significantly longer mean/median DFS for amelanotic vs. melanotic metastases. Melanogenesis shortens OS and DFS in patients with metastatic melanoma. Inhibition of melanogenesis appears a rational adjuvant approach to the therapy of metastatic melanoma.

Keywords

Melanoma; Melanogenesis; Melanin; Metastatic melanoma; Survival

1. Introduction

Melanin synthesis is a multistep process of transformation of L-tyrosine and L-DOPA to the melanin polymer in a highly organized fashion involving synthesis and interaction of multiple proteins with final execution of the pathway in melanosomes, all controlled by hormonal, nutritional, and physicochemical factors [1–3]. Melanin, which protects the skin against ultraviolet radiation and reactive oxygen species, is a marker of melanocyte differentiation. In melanomas, melanogenesis can be deregulated, generating an oxidative

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microenvironment with secondary mutagenic and genotoxic effects and producing highly immunosuppressive intermediates, all potentially contributing to more aggressive cancers [1,3–5]. Melanin polymer also can attenuate the effectiveness of radiotherapy and chemotherapy because of its scavenging properties [1,6].

We evaluated the relationship between melanogenesis and clinical outcome in 73 patients with cutaneous melanoma (American Joint Committee on Cancer [AJCC] stages I–III), who were treated in the Oncology Centre, Prof. Franciszek Łukaszczyk Memorial Hospital, Bydgoszcz, Poland during the period 2003–2009.

2. Materials and methods

The samples consisted of primary melanomas (20 AJCC stage I, 24 stage II, and 29 stage III, consisting of 2 acral, 39 nodular, and 32 superficial spreading cancers) and 42 regional lymph node metastases from the same group of patients. Of this cohort, 36 (49%) developed metastases: 27 (75%) regional lymph node disease and 9 (25%) systemic metastases after a mean follow-up of 25.7 months (range 1.4–82.6 months). Stratification of melanomas was based on the percentage of cells containing melanin. Melanomas was classified as 0 (amelanotic) if melanin was either absent or present in <5% of cells, 1 if melanin was visible in 5% to 25% of melanoma cells, 2 if melanin was visible in as many as 50% of cells, and 3 (strongly pigmented) if melanin was present in >50% of cells (Fig. 1) [7]. Because melanomas classified as 1 and 2 have similarities, we assembled these two groups into a moderately pigmented melanoma group.

Clinicopathologic data were obtained from the database of the Oncology Center and the date of deaths from the Department of Registry Office in Bydgoszcz, Poland. Overall survival (OS) and disease-free (DFS) survival analysis was performed using the log-rank test. Differences between mean/median OS and DFS (days) were analyzed using the Student *t* test. $P < .05$ was considered statistically significant. All statistical analyses were performed using Prism 5.0 (GraphPad Software, San Diego, CA).

3. Results

In the group of melanotic melanomas, melanin was located predominantly in the tumor cells in 25 cases (59.5%) and in macrophages in 13 cases (31.0%). In 4 cases (9.5%), melanin was located evenly in melanoma cells and macrophages. In 24 cases (57.1%), melanoma cells with melanin were scattered, and in 18 (42.9%), they were distributed focally.

No relationship between necrosis and melanogenesis was found. Necrosis was seen in both amelanotic and melanotic melanomas in 8 of 31 (25%) and 14 of 42 (33%), respectively. In two cases of amelanotic melanomas and in three cases of melanotic melanomas, melanophages surrounded necrotic regions, but the amount of melanin was not greater than in melanomas without necrosis. In addition, no significant differences in mitotic activity or Ki-67 labeling index were found between the amelanotic and melanotic groups.

Comparing OS and DFS in amelanotic and melanotic disease in all stages did not reveal significant differences (Fig. 2A and B). However, if only primary melanomas giving rise to metastases were compared, melanotic melanomas exhibited significantly shorter DFS and OS than amelanotic cancers (Fig. 2C; $\chi^2 = 7.554$, $P = .0229$, log-rank [Mantel-Cox] test; amelanotic vs. strongly pigmented: $\chi^2 = 6.113$, $P = .0134$ [log-rank test], $\chi^2 = 6.570$, $P = .0104$ [Gehan-Breslow-Wilcoxon test]; amelanotic vs. moderately pigmented: $\chi^2 = 5.656$, $P = .0174$ [log-rank test], $\chi^2 = 4.264$, $P = .0388$ [Gehan-Breslow-Wilcoxon test]). Mean/median OS (days) for metastasizing amelanotic melanomas (946.8/746.0) was significantly longer ($P = .0456$) than for melanin-producing melanomas (638.8/546.0). Similarly,

melanin-producing lymph node metastases showed shorter OS (FIG. 2D; log-rank test for trend, $\chi^2 = 3.972$, $P = .0463$; amelanotic vs. strongly pigmented: $\chi^2 = 6.603$, $P = .0102$ [log-rank test], $\chi^2 = 5.127$, $P = .0236$ [Gehan-Breslow-Wilcoxon test]). Again, the mean/median OS for amelanotic metastases (920.0/748.0) was significantly longer ($P = .0095$) than for melanotic metastases (521.5/525.0). Moreover, melanin production in lymph node metastases was associated with a significantly shorter DFS (Fig. 2E; log-rank [Mantel-Cox] test, $\chi^2 = 11.43$, $P = .0033$, $\chi^2 = 8.864$, $P = .0029$ [log-rank test for trend]; amelanotic vs. moderately pigmented: $\chi^2 = 10.23$, $P = .0014$ [log-rank test], $\chi^2 = 8.770$, $P = .0031$ [Gehan-Breslow-Wilcoxon test]; amelanotic vs. strongly pigmented: $\chi^2 = 7.812$, $P = .0052$ [log-rank test], $\chi^2 = 6.117$, $P = .0134$ [Gehan-Breslow-Wilcoxon test]). This is further illustrated by the significantly longer ($P = .0135$) mean/median DFS for amelanotic metastases (206.0/122.0) vs. melanotic ones (15.8/0.0). When matched primary and metastatic melanotic melanomas were stratified into tumors with higher and lower melanin pigmentation, the DFS was significantly shorter in the group in which melanin pigmentation was increased in metastases (Fig. 2F; log-rank [Mantel-Cox] test, $\chi^2 = 4.071$, $P = .0436$).

4. Discussion

Because melanogenesis can affect immune responses to melanoma and influence responses to chemotherapy and radiotherapy, we analyzed OS and DFS in melanoma patients in relation to the extent of tumor pigmentation. Our data showed that patients with metastatic melanotic melanomas had shorter DFS and OS than those with amelanotic tumors (Fig. 2). A similar relation was observed for lymph node metastases, with significantly shorter OS and DFS for melanin-producing lymph node metastases.

Melanoma, which is increasing in incidence in white populations, has a high mortality rate [8], despite recent advances in targeted therapy [9–11]. The latter is ultimately ineffective because of the development of resistance [9,11]. In this study, we found that in metastatic melanomas, production of pigment adversely affect the clinical outcome. Specifically, metastatic melanotic melanomas were associated with shorter DFS and OS than amelanotic tumors. However, the lack of significant differences in OS and DFS between amelanotic and melanotic tumors when patients with localized and metastasizing melanomas were pooled requires explanation. In particular, in primary melanomas giving rise to metastases, such differences were observed. Thus, in localized melanomas, OS and DFS are affected by both melanogenesis and other factors, which remain to be determined.

Concerning melanin synthesis, it can slow tumor growth if it is not deregulated because of the metabolic load on the cells [1,2]. However, when the process is deregulated or involves pheomelanogenesis, the growth of tumor will increase, putatively because of the production and release of mutagenic and reactive intermediates of melanogenesis [1,2]. In support of this concept, Mitra et al [12] demonstrated an ultraviolet radiation-independent pathway to melanoma carcinogenesis in mice that was accelerated by pheomelanogenesis [12]. Therefore, in advanced disease (stages III and IV), melanogenesis had a significant impact on OS and DFS. Thus, our findings and the recent report by Mitra et al [12] corroborate the view that melanogenesis can be a pathogenic factor in melanoma progression, affecting tumor behavior and interacting with host factors such as immunity, which results in the creation of an immunosuppressive and mutagenic environment [1,3,8,13].

In conclusion, melanogenesis shortens OS and DFS in patients with metastatic melanomas, which suggests inhibition of melanogenesis as an adjuvant approach to the therapy of melanotic melanomas.

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Fig. 1.
Representative sections of amelanotic (A), moderately (B, two cases separated by dashed line), and strongly (C, two cases separated by dashed line) pigmented melanomas. Scale bar, 50 μ m.

Fig. 2.

Relationship between melanin content and disease-free and overall survival in patients with melanomas. A and B, DFS (A) and OS (B) curves in all melanoma patients (localized and metastatic disease) were not affected by melanogenesis. C and D, Melanogenesis in primary metastasizing melanomas (C) and lymph node metastases (D) correlated with significantly shorter OS. E, The presence of melanin in lymph node metastases also significant correlates with shorter DFS. F, When stratified into groups with decreased or increased melanin content in metastases relative to primary melanomas, there was a significant correlation with DFS.