Prognostic Molecular Markers in Melanoma

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Emilia Hugdahl, dermatologist at Haukeland University Hospital, defended her thesis in April 20, 2018: "Prognostic and molecular markers in primary and metastatic cutaneous melanoma" for the degree of PhD at University of Bergen, Norway. Opponents were Johan Hansson, Stockholm, Silje Fismen, Tromsø, and Leiv M. Hove, Bergen. Supervisors were Lars Akslen and Rita Grude Ladstein.

The global incidence of cutaneous melanoma has increased tremendously during the last decades. The course of melanoma progression is largely unpredictable, and despite recent progress in treatment opportunities, many patients with advanced melanoma do not benefit from these. To improve prognostication and find new potential treatment targets, a deeper understanding of molecular alterations, basic tumor and microenvironment biology and tumor heterogeneity is needed.

In this PhD project, we wanted to explore markers related to both tumor cell-specific and tumor microenvironment properties in primary and matched metastatic lesions, in order to improve our understanding of tumor heterogeneity, prognostication, and possibilities for improved targeted treatment.

In the 3 separate studies, we examined a patient series consisting of 255 consecutive cases of primary nodular cutaneous melanoma diagnosed at the Department of Pathology, Haukeland University Hospital (Bergen, Norway) during 1998–2008. In Paper II and III, 78 paired biopsies from the first appearing local (skin; $n=26$) or regional metastatic tumor (lymph nodes; $n=52$) were examined in addition to the primary tumors (2, 3). In Paper I, BRAF-V600E and total BRAF expression were assessed by immunohistochemistry using TMAs ($n=248$), and mutation status was assessed by real-time PCR ($n=191$) (1). In Paper II, *TERT* promoter mutation status was assessed by Sanger sequencing (194 primary tumors and 72 metastases), and TERT protein expression by immunohistochemistry using TMAs (248 primary tumors and metastases) (2). In Paper III, vascular proliferation index (VPI) was assessed by dual immunohistochemistry using Factor-VIII/Ki67 staining (in 242 primary melanomas and 69 metastases (3). Expression of UPAR and HSP27 was determined by immunohistochemistry using TMAs (248 primary tumors and 68 metastases), and selected histopathologic features of the metastases were recorded based on HE-slides ($n=73$) (3).

Fig. 1. From left to right: Johan Hansson (Opponent), Silje Fismen (Opponent), Svein Helland (acting Dean), Emilia Hugdahl (Respondent) and Leiv M. Hove (Opponent).

Positive BRAF-V600E expression was present in 35 of the cases and was significantly associated with increased tumor thickness, presence of tumor ulceration and reduced survival. There was 88% concordance between BRAF-V600E expression and mutation status (I).

TERT mutations were present in 68% of primary melanomas and 64% of metastases (2). The mutation status was discordant between primary tumor and metastasis in 24%, among which 71% did not show a mutation in the metastatic tumor. *TERT* mutated tumors showed no significant association with reduced survival, neither in primary melanoma nor in metastases. TERT protein expression did not correlate with mutation status, but positive TERT expression in primary melanomas was significantly associated with increased tumor thickness and with reduced survival (II).

High VPI and high UPAR expression were associated with each other, and with aggressive tumor features and reduced survival in primary melanoma. In loco-regional metastases, high UPAR expression was significantly associated with increased MVD, but no prognostic value was found for any marker of angiogenesis, including UPAR and HSP27. Presence of tumor necrosis in loco-regional metastases, as a marker of tumor hypoxia, was associated with increased tumor size, high VPI, high HSP27 expression and reduced survival. Among paired primary and metastatic tumors, median MVD was significantly higher in metastases, while the opposite was found for VPI, which had significantly lower median value in metastases. Among discordant VPI cases, most had lower VPI in metastases compared to primary tumors. The same pattern was seen for UPAR expression, while among discordant cases for necrosis, the majority showed a switch from absent in the primary to present in the metastasis (III).

In conclusion, a prognostic value was found for BRAF-V600E protein expression, TERT protein expression, VPI and UPAR expression in primary melanoma, and for tumor necrosis in loco-regional metastases. Inter-tumoral discordancy of *TERT* mutation status was demonstrated.

List of publications

I. Hugdahl E, Kalvenes MB, Puntervoll HE, Ladstein RL, Akslen LA. BRAF-V600E expression in primary nodular melanoma is associated with aggressive tumour features and reduced survival. Br J Cancer 2016; 114: 801–808.
II. Hugdahl E, Kalvenes MB, Mannelqvist M, Ladstein RG, Akslen LA. Prognostic impact and concordance of TERT promoter mutation and protein expression in matched primary and metastatic cutaneous melanoma. Br J Cancer 2018; 118: 98–105.
III. Hugdahl E, Bachmann IM, Schuster C, Ladstein RG, Akslen LA. Prognostic value of uPAR expression and angiogenesis in primary and metastatic melanoma. PLoS One 2019; 14: e0210399.