Molecular mysteries of melanoma:
Markers of conjunctival melanoma

On December 4, 2020, Lauge Hjorth Mikkelsen defended his thesis, "Molecular Biology in Conjunctival Melanoma and the Relationship to Mucosal Melanoma," at the Depts. of Pathology and Ophthalmology, Copenhagen University Hospital. The main supervisor was Professor Steffen Heegaard, MD, DMSc., Depts. of Pathology and Ophthalmology, with co-supervisors Peter Bjerre Toft, MD, DMSc., and Ann-Cathrine Larsen, MD, Ph.D at the Dept. of Ophthalmology as well as Mette Klarskov Andersen, MD, DMSc., and Karin Wadt, MD, Ph.D. at the Dept. of Clinical Genetics at Rigshospitalet, Copenhagen University Hospital.

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Ophthalmologists encounter three melanoma subtypes: skin, uveal, and conjunctival. Whereas skin and uveal melanoma have been extensively studied, less is known about the pathogenesis of conjunctival melanoma. Conjunctival melanoma is a rare ocular surface tumor with an annual incidence in the Nordics of 0.5 cases/million. With proper treatment, the course of the disease is often simple, but metastases can drastically worsen the prognosis. As it is a mucosal melanoma, we decided to molecularly study conjunctival melanoma compared to other mucosal melanomas, e.g., anorectal, sinonasal, and urogenital. However, conjunctival melanoma is the only mucosal melanoma exposed to sunlight.
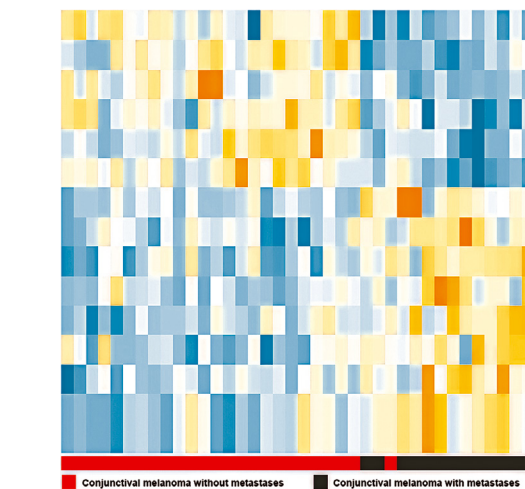
We investigated the distribution of mutations in around 150 known cancer genes in conjunctival and other mucosal melanomas. We found that 25% of conjunctival melanomas showed mutations in *BRAF*, a gene frequently mutated in skin melanoma. Mutations in this gene allow cells to proliferate rapidly through activation of the MAPK-pathway, the most important signaling pathway in melanoma. However, other mucosal melanomas did not have mutations in *BRAF* and were much more heterogeneous, suggesting other genetic drivers. Our findings indicate that conjunctival melanoma is a distinct subtype of mucosal melanoma.

In addition to mutations, we also investigated gene expression. Many mechanisms can affect gene expression, and changes can cause over- or underexpression of gene products. The disruption in the normal molecular signaling pathways can lead to cancer. We found 41 genes that were significantly deregulated in conjunctival melanoma but not in other mucosal melanomas. Interestingly, many of these genes were important for immune function, such as *PD-L1*, which was highly upregulated in conjunctival melanoma. *PD-L1* is important in cancer immunology. Novel drugs inhibiting PD-L1's interaction with tumor cells have shown promising results in many cancers, including skin melanoma. Our findings support the potential for these drugs to be used in conjunctival melanoma but not in other mucosal melanomas. We tested whether the differences could be explained by sun exposure but were unable to confirm this.

Lastly, we assessed whether microRNA (miRNA) expression could be used to predict metastasis of conjunctival melanoma. We compared the miRNAs profile of conjunctival melanoma with and without metastatic spreading and found that the groups could be separated by the expression of 15 miRNAs. No single miRNA was found to be prognostic for metastasis. This could be because of the limited sample size due to the rareness of the disease.

Color Key

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Figure 1. MicroRNA expression in metastatic and non-metastatic conjunctival melanoma. The two groups could be classified based on the expression of 15 miRNAs, 6 downregulated and 9 upregulated, in metastatic conjunctival melanoma. Adapted from Mikkelsen LH, et al. Global microRNA profiling of metastatic conjunctival melanoma. Melanoma Res. 2019 Oct;29(5):465-473.

Key points:

- Conjunctival melanoma is genetically different from the other mucosal melanomas, as shown by frequency of BRAF mutations.

- Conjunctival melanoma has a specific gene expression with more PD-L1 than other mucosal melanomas. This may be therapeutically beneficial in the future.

- Conjunctival melanoma with and without metastasis can be classified based on the expression of 15 miRNAs. No single miRNA could predict metastatic behavior.

Articles in the dissertation

1. Mikkelsen LH, et al. Mucosal malignant melanoma - a clinical, oncological, pathological and genetic survey. *APMIS.* 2016;124(6):475-86.
2. Mikkelsen LH, et al. Global microRNA profiling of metastatic conjunctival melanoma. *Melanoma Res.* 2019 Oct;29(5):465-473.
3. Mikkelsen LH, et al. The molecular profile of mucosal melanoma. *Melanoma Res.* 2020 Dec;30(6):533-542.