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GENETIC AND EPIGENETIC ALTERATIONS IN MELANOMA

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ABSTRACT

Malignant melanoma is a disease that may arise in several different parts of the body, preferentially the skin, rarely in the mucosal membranes or the choroidal tissues of the eye. The incidence of cutaneous melanoma (CMM) is steadily increasing in the Caucasian populations, unlike uveal melanoma (UM) that shows a stable incidence. The increase is likely to be related to t UV-irradiation leading to genetic aberrations that allow skin melanocytes to develop unlimited growth and immortality and ultimately lead to metastases.

Paper I presents a genomic and epigenomic screening of 77 metastatic cutaneous melanoma metastases for the protein expression of $p16^{INK4A}$ in relation to 3 well-known causes of expression loss: truncating and non-synonymous mutations in *CDKN2A*, the gene for p16INK4A, transcriptional silencing of $p16^{INK4A}$ gene promoter and previously studied deletions in the *CDKN2A* loci encompassing $p16^{INK4A}$. These aberrations were compared to $p16^{INK4A}$ expression in tumours and presence of mutations in *BRAF* and *NRAS* genes. Unexpectedly, a significant association between tumours carrying *NRAS* mutations and transcriptional silencing of $p16^{INK4A}$ promoter was observed.

Paper II was a case study of a family with multiple cases of uveal melanoma. Family members with were found to be negative for germ-line *CDKN2A* aberrations, why next generation sequencing was employed. The proband, the proband's sister and both parents were analyzed. The final mapped and filtered variants were filtered against variants found in the DNA of the non-carrier mother. A germ-line, frame-shift, insertion in *BAP1* (exon 3 c.75insG) was identified and validated by Sanger sequencing. The insertion leads to a truncation at codon 43 and was found to segregate with the disease.

Paper III is a retrospective study to evaluate the naturally occurring transcriptional silencing of DNA repair protein O^6-methylguanine DNA methyltransferase, *MGMT*. MGMT activity counteracts the efficacy of alkylating chemotherapy. Two cohorts of patients mainly derived from Sweden (n=74) and Belgium (n=79) were included, in total encompassing 191 tumours. The hypermethylation of *MGMT* gene promoter was found in 21.5% of tumours successfully analyzed (28 positive, 130 total) and to be associated with a significantly longer progression free survival (PFS) and to be an independent variable in a multivariate analysis for PFS.

Paper IV is an in vitro melanoma study for combination therapy efficacy. The BRAFV600E- melanoma cell line A375 and a mutant BRAF inhibitor (BRAFi)-resistant subline were subjected to sequential and simultaneous exposures for BRAFi PLX4720 and temozolomide (TMZ). Administration order was found to influence the treatment outcome: administration of BRAFi followed by TMZ displayed a poorer efficacy compared to exposure simultaneously or administration in the reverse order. This effect was related to BRAFi induction of *MGMT* mRNA and protein, but also induction of the DNA damage marker γH2AX by BRAFi and TMZ.