Efficient immunomagnetic isolation of melanoma cells with diverse genetic backgrounds from blood

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Background

Melanoma is a highly aggressive cancer and until recently, the majority of patients with visceral metastases had survival rates of less than one year.

Targeted treatment has revolutionized melanoma therapy with BRAF and MEK inhibitors tailored to BRAF mutant melanomas producing remarkable responses until resistance, caused by various mechanisms, occurs.

The potential use of circulating tumor cells (CTCs) in melanoma to derive tumor-bioinformation for such targeted treatment and importantly monitor these cells for emerging resistance biomarkers requires effective strategies of melanoma CTC isolation, however melanoma tissues display high intra- and inter-patient heterogeneity, which also affects cell surface protein expression.

Aim

To efficiently isolate circulating melanoma cells of various genotypes.

Methods

A panel of mouse monoclonal antibodies against melanoma cell surface antigens were tested for suitability for immunomagnetic melanoma cell capture:

- Antibody interaction with a cohort of eight different melanoma cell lines, as well as the lack of interaction with lymphocytes was determined by *immunocytostaining* and *flow cytometry.*
- Anti-MCAM, anti-HMW-MAA and anti-LHM3 antibodies were selected for further analysis.
- Melanoma cells were spiked into healthy donor blood lymphocytes and isolated with combinations of the selected antibodies using the *IsoFlux CTC isolation platform and the "Rare Cell Enrichment Kit"* (Fluxion).
- Testing of these antibody combinations on patient samples is underway.

Melanoma cell lines

Cell Line	Characteristics / Genotype
SkMel28	$BRAF^{V600E}$, $CDK4^{R24C}$, $EGFR^{P753S}$, $PTEN^{T167A}$,$PTEN^{R130Q}$, $p53^{L145R}$, express wild type $p16^{INK4a}$, epithelial expression signature
501mel	$BRAF^{V600E}$,$CTNNB1^{D32H}$, $CTNNB1^{D37F}$, p16-null, EMT expression signature
NM176	$BRAF^{V600E}$, $CDK4^{R24C}$, express wild type $p16^{INK4a}$, high melanin expression
A375	$BRAF^{V600E}$, $p16INK4a^{E61*}$, $p16INK4a^{E69*}$
WMM1175	$NRAS^{G13R}$, p16-null, p53-null
MelRM	$NRAS^{Q61R}$
MelMS	c-$Kit^{W557\text{-}K558del}$
M230	c-Kit^{L576P}

Table 1:
Eight melanoma cell lines with different genotypes were included in the study. Additionally, the lymphocyte cell line WME-099 was tested to exclude antibody interaction with blood cells.

Melanoma cell surface protein expression is heterogeneous

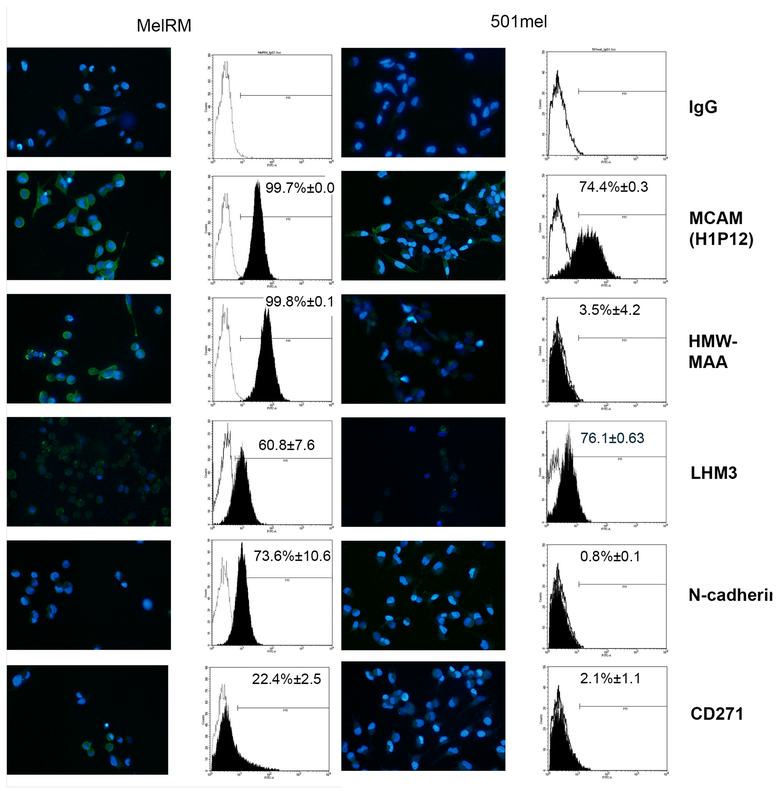

Figure 1: **Immunocytostaining and FACS analysis**
The cohort of melanoma cell lines and WME-099 lymphocytes were incubated with the indicated antibodies and antibody presence on the cells detected with green fluorescently labelled secondary antibody. Antibody interaction with the cells (proportion of cells and intensity of staining) was determined by microscopy or flow cytometry. Representative images for MelRM and 501mel cells are depicted.

Melanoma cell surface protein expression is heterogeneous

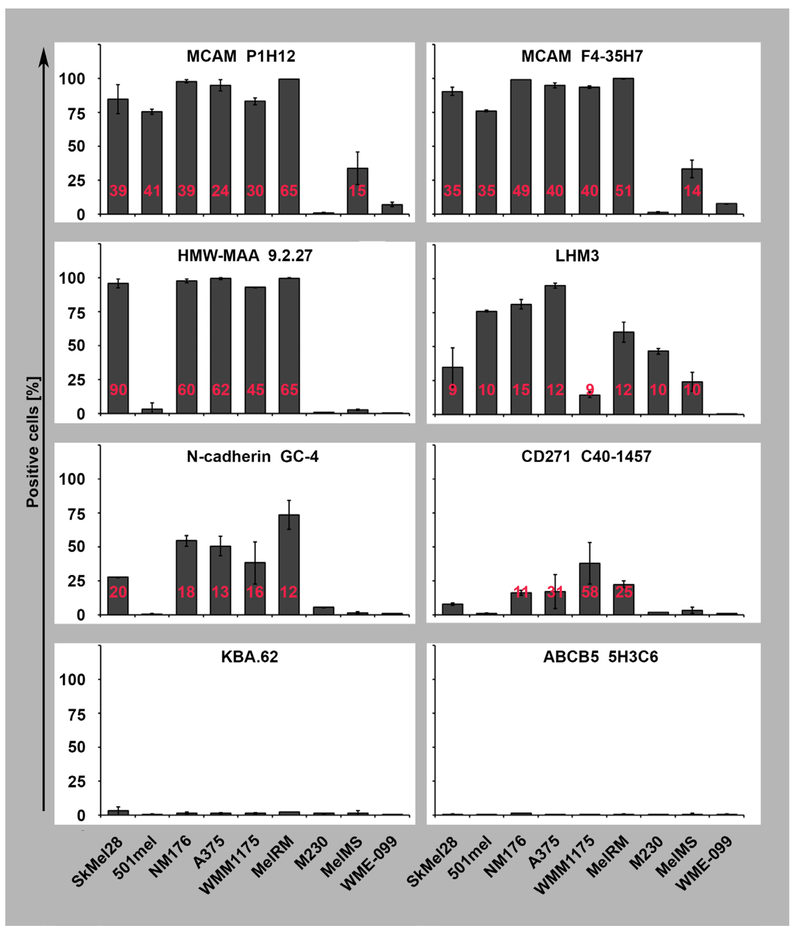

Figure 2: FACS analysis was performed as described above and the proportion of cells interacting with the indicated antibodies is shown. The average mean fluorescent intensity indicating the antibody affinity to the cells is shown in red for samples with >10% positive cells.

Antibodies for melanoma cell identification

Cell line	Anti-MelanA	Anti-S100-b	Anti-Gp100	All combined
SkMel28	+++	+++	+++	+++
501mel	+++	+-	+	+++
NM176	+++	++(-)	++	+++
A375	-	+-	++(-)	++(+)
WMM1175	-	+-	++(+)	++(+)
MelRM	+-	++(+)	-	+++
MelMS	+++	++(+)	+++	+++
M230	+++	++	+++	+++

Table 2: For identification of melanoma cells isolated from blood a cocktail of the shown rabbit monoclonal antibodies against melanoma associated proteins was used. Staining intensities of these antibodies with the cohort of melanoma cell lines alone and in combination is presented.

Scoring for staining intensity: *+++ highly positive; ++(+) unequal mostly highly positive; ++ positive; ++(-) unequal with a small proportion of negative cells; + detectable; +- unequal mostly detectable staining, - negative*

Isolation of melanoma cells

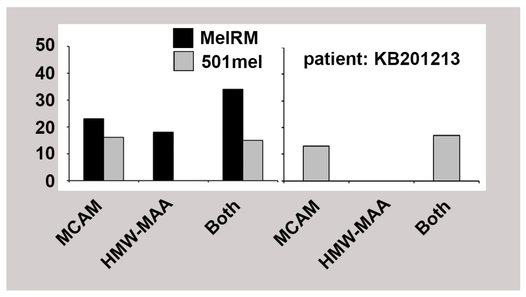

Figure 3: Tumor cell capture with the indicated antibodies alone or in combination using the "Rare Cell Enrichment Kit" and the IsoFlux platform (Fluxion). **Left:** 50 melanoma cells were spiked into healthy donor blood lymphocytes. **Right:** CTCs from a $BRAF^{V600E}$ melanoma patient's blood.

Conclusions

- Cell surface protein expression on melanoma cells is highly heterogeneous.
- MCAM and HMW-MAA are the common cell surface proteins on melanoma cells.
- Previous strategies, which targeted only MCAM for melanoma cell capture and the anti-HMW-MAA antibody for melanoma cell identification would miss CTCs from melanoma patients with 501mel like phenotype and we propose combination of both antibodies for immuno-magnetic cutaneous melanoma CTC isolation.
- Melanoma CTC identification can be performed with a cocktail of melanoma specific antibodies.
- CTCs of c-Kit mutant melanoma (usually of acral or mucosal origin) may be particularly difficult to isolate due to very limited MCAM expression and from our data only LHM3 emerged as a potential cell surface antigen with some promise for isolation of these rare melanoma cells.

Acknowledgements

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