BioMed RESEARCH GRAZ

Cell GRAZ Bank

Medical University of Graz

MUG-Mel2, a novel highly pigmented and well characterized NRAS mutated human melanoma cell line

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NRAS mutation in melanoma has been associated with aggressive tumor biology and poor prognosis. Although targeted therapy has been tested for NRAS mutated melanoma, response rates still appear much weaker, than in BRAF mutated melanoma. While plenty of cell lines exist, however, only few melanogenic cell lines retain their *in vivo* characteristics. In this work we present an intensively pigmented and well-characterized cell line derived from a highly aggressive NRAS mutated cutaneous melanoma, named MUG-Mel2. We present the clinical course, unique morphology, angiogenic properties, growth characteristics using *in vivo* experiments and 3D cell culture, and results of the exome gene sequencing of an intensively pigmented melanogenic cell line MUG-Mel2, derived from a cutaneous metastasis of an aggressive NRAS p. Q61R mutated melanoma. Amongst several genetic alterations, mutations in GRIN2A, CREBP, PIK3C2G, ATM and ATR were present. These mutations, known to reinforce DNA repair problems in melanoma, might serve as potential treatment targets. The aggressive and fast growing behavior in animal models and the obtained phenotype in 3D culture reveal a perfect model for research in the field of NRAS mutated melanoma.

Patient History and Establishment of the Cell line MUG-Mel2

The MUG-Mel2 cell line was obtained from a cutaneous primary, ulcerated melanoma on the left shoulder. Clinical course time line is described in Figure 1. Four months after wide surgical excision of the primary melanoma, the patient developed multiple cutaneous satellite metastases around the scar with progressive spread all over the body (Fig. 2 A+B).

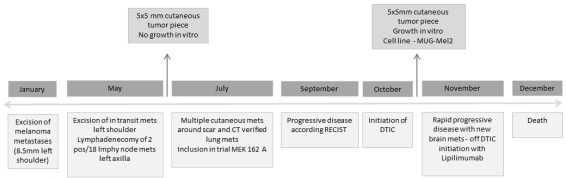

Figure 1: Clinical course and date of MUG-Mel2 cell line establishment

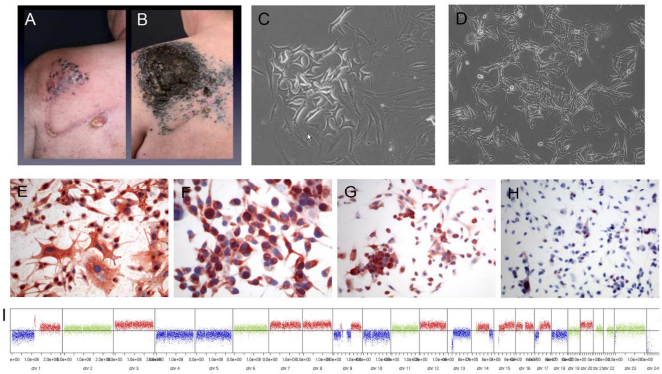

Figure 2: Male patient with cutaneous metastases appearing shortly after wide surgical excision of a primary melanoma on his left shoulder (A+B), outgrowth of melanoma tumor cells surrounded by stroma cells (C), MUG-Mel2 cell line after ten days (D). Immunohistochemistry revealed in a strong HMB-45 expression (E), strong Melan-A expression (F) strong Tyrosinase expression (G) and a weak S-100 expression (H). Copy number variation profile of MUG-Mel2 passage 89.(I).

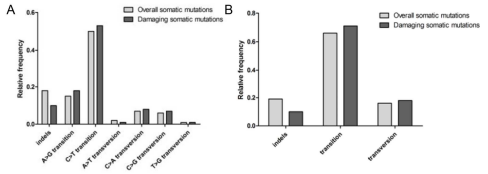

Figure 3: Relative frequencies of each type of nucleotide substitution (A) and of indels, transitions and transversions (B) among the overall somatic mutations and among the damaging somatic mutations detected in MUG-Mel 2 cell line.

Growing under different conditions

The ability of MUG-Mel2 to form spheroids could be achieved by cultivation in NFC scaffolds. Sterile GrowDex™ nanofibrillar cellulose (NFC) hydrogel was obtained from UPM-Kymmene Corporation, Finland. The NFC concentration of the hydrogel was 1.55 wt%. To determine the optimal conditions for 3D cell culturing, three concentrations (0.4%; 0.7% and 1% wt/v) were used. Figure 4: Morphology of MUG-Mel2 in 3D cultures. Cells formed spheroids in nanofibrillar cellulose (NFC) after two days in a concentration of 0.4% (A: 50x magnification), after five days in a concentration of 0.4% (B: 50x magnification); intensive brown staining was observed. Calcein staining (green) revealed the viability of the spheroids, nuclei were counterstained with DAPI (blue) (C).

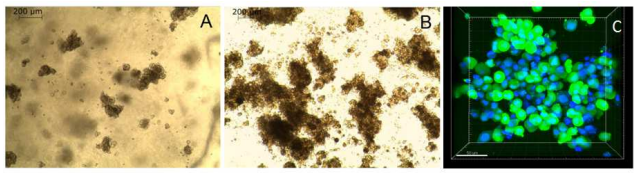

Figure 4: 3D - Culturing in Nanofibrillar Cellulose (NFC)

Ex ovo CAM Assay

Fertilized Lohmann white classic chicken eggs from local hatchery were incubated at 37.6 ℃ and 70% humidity. Macroscopic observation revealed attraction of numerous vessels that developed radially towards the onplants and the presence of loco-regional metastases with solitary evading tumor cells surrounded by newly formed blood vessels (Fig. 5A-C). HE staining revealed that solid tumor samples were strongly connate to the CAM with outgrowth of the tumor invading avian vasculature (Fig. 5D).

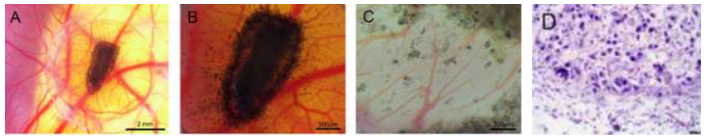

Figure 5: *ovo* CAM assay: MUG-Mel2 onplants formed highly pigmented tumors within the silicone ring on the CAM surface after three days of incubation.

Xenotransplants confirmed tumorigenicity of MUG-Mel2

The melanoma cells were easily detectable because of the intense pigmentations. To determine tumorigenicity of MUG-Mel2, cells were injected first into NOD/SCID/Il −2rznull (NSG) mice. All three (3/3) xenotransplants developed black nodules after 10 days. The same evolution and growth was also detectable in all xenotransplants (5/5) in nude mice at 100%. To verify the melanoma growing in mice, IHC were performed.

Figure 6: Tumorigenicity: MUG-Mel2 growing in NSG mice (A), black tumor was visible within 10 days (B). Separated tumor from nude mouse (C). IHC from xenotransplantations with strong HMB-45 expression (D), strong Melanin-A expression (E).

To conclude, the newly established cell line MUG-Mel2 presented due to its phenotypically and genotypically precise characterization, aggressive and fast growing behavior in mouse and chick models and in particular, with the high pigmentation a promising new model for innovative NRAS therapy options.