Exploring Tenascin-C in MAPKi resistance in BRAFV600-mutant melanoma

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Objective

Melanoma patients harboring a BRAFV600 mutation benefit from small molecule inhibitors that selectively target BRAF signaling, yielding a rapid initial response. However, approximately 50% of patients eventually become resistant to these therapies. These resistance mechanisms can be intrinsic or acquired, posing a significant challenge to long-term therapeutic efficacy. In previous proteomics analyses we have identified several proteins that differentiate between responders and non-responders to MAPK inhibitors (MAPKi), including PTRF (Paulitschke V, et al. 2019) and Tenascin-C (TNC). TNC, a matricellular protein, has been associated with various cancers, although its role in melanoma is still under investigation.

Methods

This study aims to evaluate the potential role of TNC in MAPKi resistance and melanoma progression. Using primary BRAF-mutant melanoma cells, we generated TNC knockout cells via CRISPR/Cas9 gene editing, using two different guide RNAs used for the knockout and two for the scrambled control. Having confirmed the destruction of the gene segment by sequencing and the absence of protein expression by proteomics, we cultivated single-cell sorted clones of two individual knockout (KO) cells (TNC216 and TNC414) and two individual scrambled control (CTRL) cells (SCR111 and SCR211). We then performed six independent assays to evaluate the potential implications of TNC in melanoma cell proliferation and migration. Using the PHIO Cellwatcher device (https://www.phio.de), we analyzed the cells every 30 minutes over a period of 40 hours. Figure 1 illustrates our study approach.

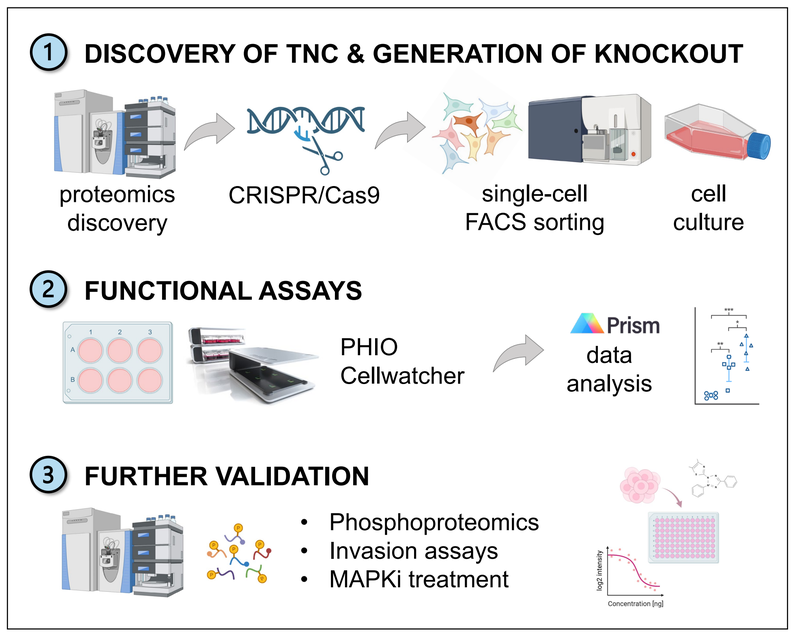

Figure 1: Study approach.
Following the proteomic discovery of TNC, TNC-knockout cells were created using primary BRAF-mutant melanoma cells and CRISPR/Cas9 gene editing. Single-cell sorted clones were cultivated and used for functional assays to analyze the proliferation and motility of the knockout (KO) cells compared to the control (CTRL) cells. Further validation, including phosphoproteomics, will follow.

Results

Results from proliferation and motility assays, conducted using the PHIO Cellwatcher device (https://www.phio.de), showed significant differences between TNC knockout (KO) and scrambled control (CTRL) cells. After normalizing the data, we analyzed cell proliferation based on the growth rate, and cell motility over a 5–30 hour timeframe during the experiments. We performed a two-way ANOVA for multiple comparisons and an unpaired t test. The growth rate, which represents cell proliferation, was significantly altered between the KO and CTRL cells, but not within the KO and CTRL groups (Figure 2A).

This effect remains consistent, when the data from the individual cells is pooled (Figure 2B). Furthermore, cell motility is also significantly different between the KO and CTRL cells, but not within the KO and CTRL groups (Figure 2C). This effect is retained when the data are pooled (Figure 2D). A cartographic representation of the cells over time, visualized by arrow maps for the time period 5–30 hours, illustrates the differences between the KO and CTRL cells (Figure 2E). Overall, these results suggest that TNC influences cellular processes such as proliferation and motility of melanoma cells.

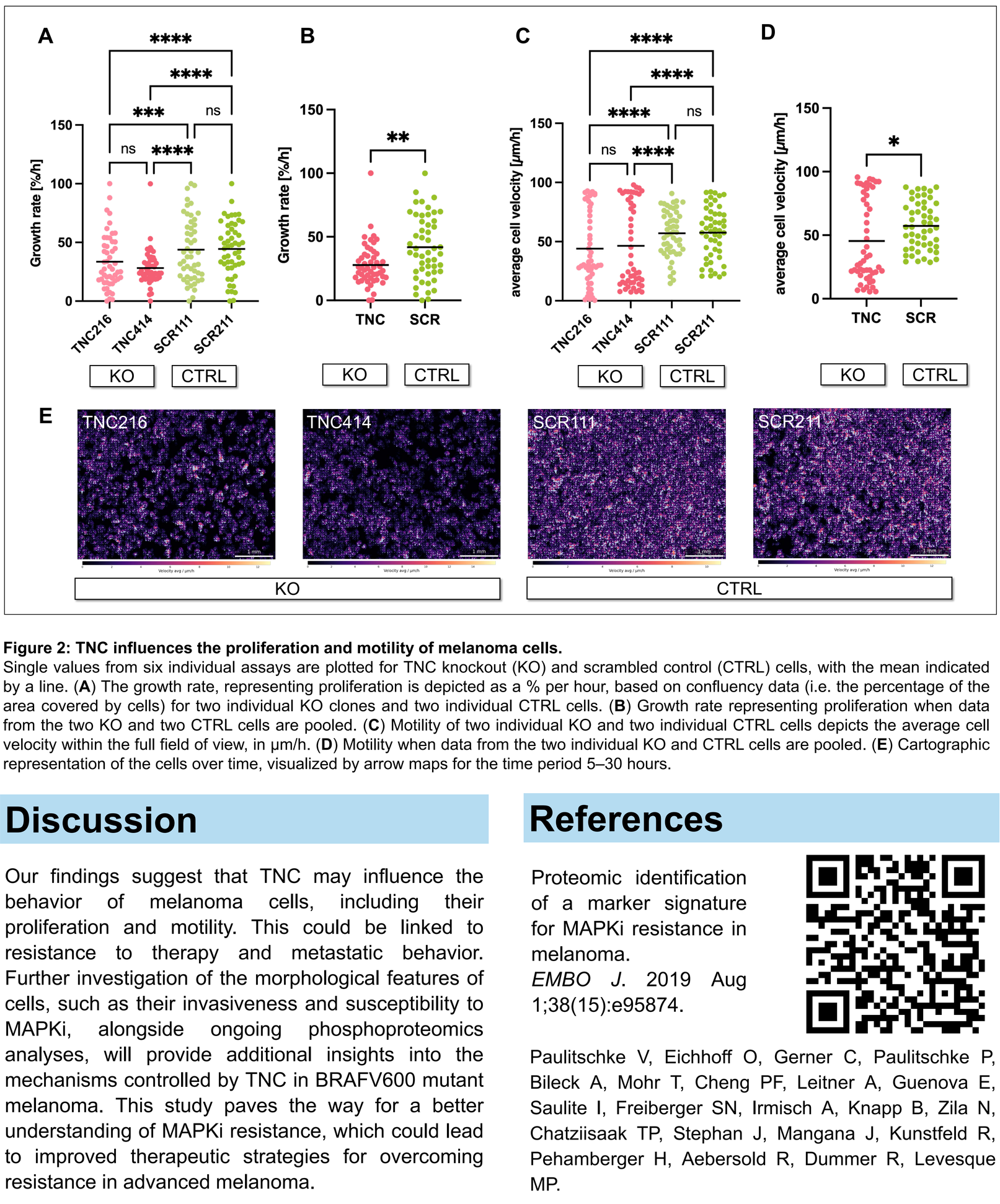

Figure 2: TNC influences the proliferation and motility of melanoma cells.
Single values from six individual assays are plotted for TNC knockout (KO) and scrambled control (CTRL) cells, with the mean indicated by a line. (**A**) The growth rate, representing proliferation is depicted as a % per hour, based on confluency data (i.e. the percentage of the area covered by cells) for two individual KO clones and two individual CTRL cells. (**B**) Growth rate representing proliferation when data from the two KO and two CTRL cells are pooled. (**C**) Motility of two individual KO and two individual CTRL cells depicts the average cell velocity within the full field of view, in µm/h. (**D**) Motility when data from the two individual KO and CTRL cells are pooled. (**E**) Cartographic representation of the cells over time, visualized by arrow maps for the time period 5–30 hours.

Discussion

Our findings suggest that TNC may influence the behavior of melanoma cells, including their proliferation and motility. This could be linked to resistance to therapy and metastatic behavior. Further investigation of the morphological features of cells, such as their invasiveness and susceptibility to MAPKi, alongside ongoing phosphoproteomics analyses, will provide additional insights into the mechanisms controlled by TNC in BRAFV600 mutant melanoma. This study paves the way for a better understanding of MAPKi resistance, which could lead to improved therapeutic strategies for overcoming resistance in advanced melanoma.

References

Proteomic identification of a marker signature for MAPKi resistance in melanoma.
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