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Targeting TRPM8: A Novel Strategy to Halt Androgen-Driven Invasiveness in Melanoma

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INTRODUCTION & AIM



Melanoma ranks among the most lethal cancers worldwide. Advanced-stage melanoma is treated with various therapeutic strategies, often accompanied by significant side effects. Recent advancements in targeted therapies, particularly those aimed at receptor tyrosine kinases and immune checkpoints, have substantially improved overall survival (OS) and long-term disease control. However, resistance mechanisms frequently develop, leading to disease progression. Consequently, the need for alternative therapeutic approaches for advanced melanoma remains pressing. The transient receptor potential melastatin-8 (TRPM8) channel has emerged as a promising molecular target implicated in the migration and proliferation of malignant cells. However, its specific role in melanoma progression Melanoma cell lines with varying malignancy degrees were treated with or without androgens in the presence or absence of newly synthesized TRPM8 modulators.

METHOD

Wound scratch and Boyden's chambers analysis were performed to evaluate cell migration and invasion. The most effective compounds were further tested in melanoma 3D spheroid models.

remains unclear.

This study aims to investigate the effects of novel TRPM8 modulators on androgen-induced migration, invasiveness, and spheroid growth in melanoma cells.





FIG. 1 ANALYSIS OF TRPM8 and AR EXPRESSION IN WM266-4 and AMM16 CELL LINES.

In **A**, lysates from metastatic melanoma WM266-4 and AMM16 cells were prepared and proteins analyzed by WB, using specific antibodies.

In **B**, protein expression levels were analyzed by densitometric analysis, using NIH Image J software. The ratio of TRPM8/Tubulin (left panel) and AR/Tubulin (right panel) was assessed.





FIG. 2 ANALYSIS OF CELL MIGRATION BY WOUND HEALING ASSAY.

The effect of TRPM8 modulators on androgen-induced migration was analyzed by wound scracth assay. Androgens promoted the migration of AMM16 and WM266-4 cells while TRPM8 modulators reversed the hormonal effect.

> 4 3

R1881

n=3

3 1

3

n=3

2



n=4

2.5

FIG.3 ANALYSIS OF MELANOMA CELLS MIGRATION AND **INVASIVENESS.**

Androgens promote migration (A and B) and invasiveness (C and D) of melanoma cells (WM266-4 and AMM16).

The addition of TRPM8 modulators (1,2, 3, 4 and 5) impairs cell migration and invasiveness.



3

2.5

2

ligrating AMM16 ce (Fold increase)

n=4



18 days

FIG. 4 THE ROLE OF TRPM8 MODULATORS IN SPHEROID SIZE GROWTH

Spheroids derived from AMM16 and WM266-4 melanoma cells in Extracellular matrix were performed. Spheroids were left untreated or treated with the synthetic androgen (10 nM R1881) in the absence or presence of different TRPM8 modulators (1,2,3 used at 100 nM) for 18 days. Changes in spheroid size and structure were monitored. Phase contrast microscopy images and data quantification (top right panel for AMM16 cell line and bottom right for panel for WM266-4 cell line) showed that the addition of hormones significantly increased the size of spheroids, while the addition of modulators abolished the androgen-induced effect.



These preclinical findings highlight TRPM8 as a promising therapeutic target in melanoma, offering potential for innovative treatment strategies to overcome limitations in current therapies.



Based on these results, the next step will be to investigate the link between AR and TRPM8.