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Implementation and Validation of a Pigmentation Model Using B16-F10 Melanoma Cells for Dermatological Applications

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INTRODUCTION

Skin **hyperpigmentation** disorders are pervasive dermatological concerns driven by dysregulated **melanogenesis**. Primary melanocytes and melanoma cell lines, used to study melanogenesis, suffer from poor reproducibility, scalability, and genetic tractability.

The murine **B16-F10 melanoma cell line** addresses these limitations through exceptional melanogenic capacity, rapid proliferation in standard culture, and high responsiveness to stimuli like 3-isobutyl-1-methylxanthine (**IBMX**).

Despite their broad application, current B16-F10 models are hindered by **key limitations**—namely poor reproducibility, limited mechanistic insight, and low throughput.

In this study, we bridge these gaps with a **multi-validated B16-F10/IBMX platform** featuring:

- **Tyrosine-optimized culture media** to standardize melanogenesis,
- **Dual chemical/genetic validation** (kojic acid + *TYR* siRNA),
- High-throughput screening of **novel sustainable actives** (*Willaertia* magna C2c Maky lysate).

MATERIALS & METHODS

Cell Culture & Media Optimization
B16-F10 melanoma cells were cultured under tyrosine-controlled conditions to ensure consistent melanogenesis. Two media types were used based on tyrosine content: high-glucose DMEM (104 µM tyrosine) for model validation and DMEM/F12 (55 µM tyrosine) for application studies. All media were supplemented with 10% FBS and antibiotics.

Treatment Protocols
Cells were treated with IBMX (10–1000 µM), kojic acid (100–200 µM), and a novel *Willaertia magna* C2c Maky lysate (0.001% w/v) across multiple experiments. Treatments were conducted for 24–48 h in 24-well plates, with vehicle controls included.

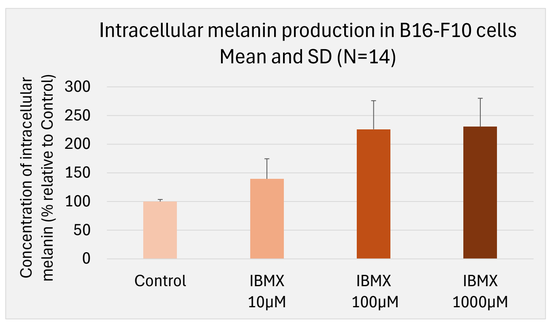
Genetic Validation
TYR gene knockdown was performed using siRNA to confirm tyrosinase-dependent melanogenesis. Transfected cells were treated with IBMX ± kojic acid, and *TYR* expression was quantified via RT-qPCR.

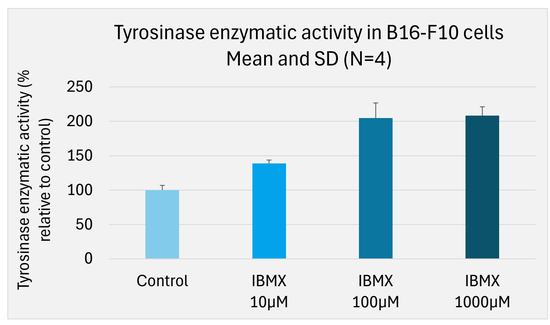
Melanin & Tyrosinase Assays
Melanin content was measured via absorbance at 405 nm using two validated extraction methods. Tyrosinase activity was assessed kinetically using L-DOPA substrate and quantified as AUC.

Statistical Analysis
Data were analyzed using Mann-Whitney U and one-way ANOVA with Tukey's post-hoc test. Significance was set at $p < 0.05$.

RESULTS

Activation of Melanogenesis in B16-F10 Cells by IBMX

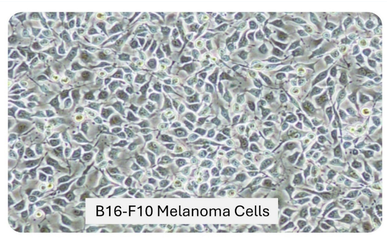


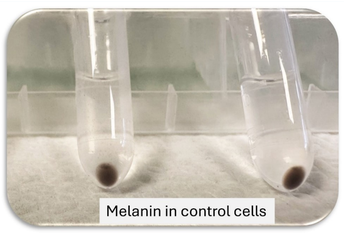


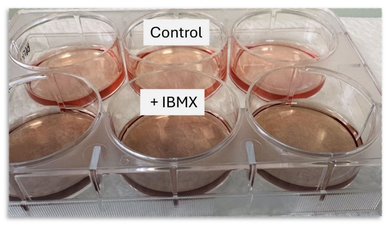
IBMX stimulation of B16-F10 cells induced strong melanogenesis in a dose- and time-dependent manner, characterized by:

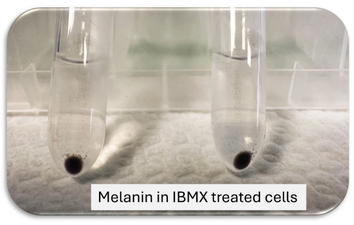
- **Increased melanin production**
- **Increased tyrosinase enzymatic activity**

Visual Evidence of IBMX-Induced Melanogenesis in B16-F10 Cells

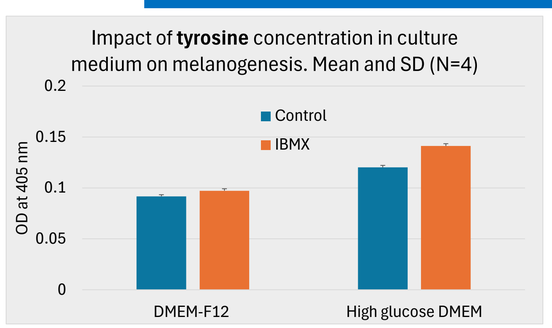






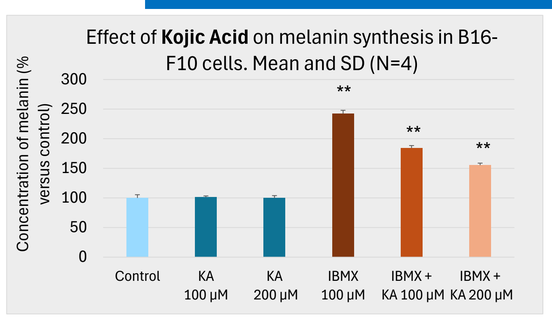


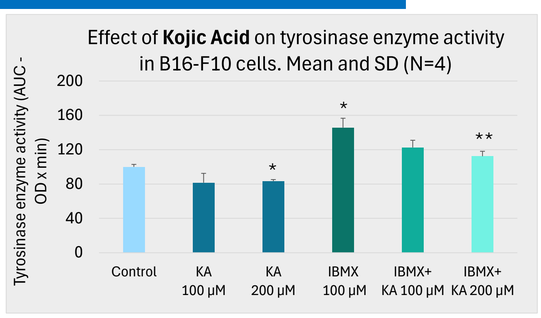
Critical Role of Tyrosine Standardization in Melanogenic Output



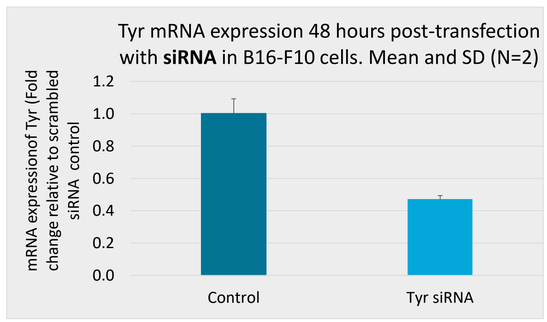
Tyrosine concentration in culture medium dictates melanogenic output in IBMX-stimulated B16-F10 cells

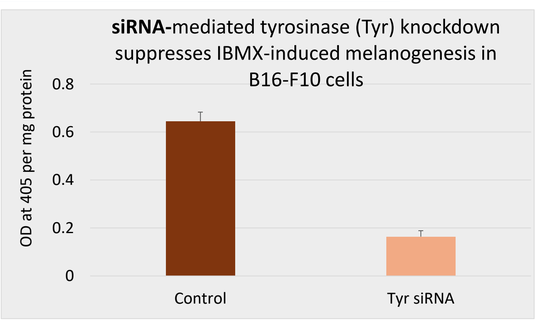
Kojic Acid Validation Benchmark



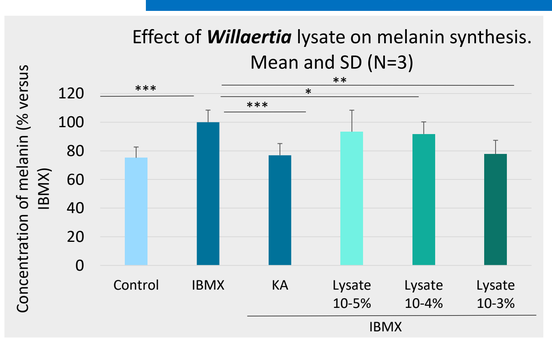


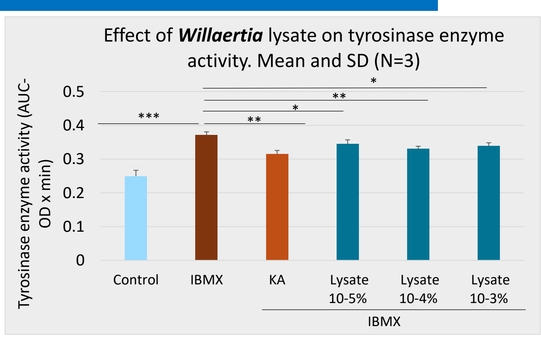
Tyrosinase Knockdown Validation via siRNA





Willaertia C2c Maky Lysate Inhibits Melanogenesis in B16-F10 Cells





CONCLUSION

We have established and validated a robust **B16-F10 model** for melanogenesis inhibition studies, highlighting the critical role of tyrosine standardization.
Using this model, we identified ***Willaertia*** lysate as a potent novel inhibitor of melanin synthesis. This discovery opens a promising avenue for developing new therapeutic agents for hyperpigmentation disorders from an unexplored biological source.