In-Vitro Toxicity of MiR-302 Precursors on Human Melanocytes and Fibroblasts

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Innovative MicroRNA Therapies

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ABSTRACT

MiR-302 is a standard marker for human pluripotent stem cells. Mello Biotech's Purified *Natural microRNA Precursor*™ F6 contains purified miR-302 precursors dissolved in Mello's proprietary F5 formulation. Extracted from Dicer-negative microRNA expressing cells, miR-302 precursors are hairpin-like small RNA molecules that can be processed to yield mature and functional miR-302 microRNAs following delivery into target cells. The mature miR-302 microrRNAs have been reported to play an important role in a wide range of stem cell-related activities, such as wound healing, tissue repairing, stem cell generation and maintenance that eventually lead to anti-aging and rejuvenation. The aim of this study is to determine the *in vitro* cytotoxic effects of miR-302 precursors on human melanocytes and fibroblasts.

SUMMARY

1) F5 formulation is non-toxic to both human melanocytes and fibroblasts.
2) F5-formulated miR-302 precursors are non-toxic to human melanocytes.
3) F5-formulated miR-302 precursors are moderately toxic to human fibroblasts.

MATERIALS AND METHODS

Human Epidermal Melanocytes and Dermal Fibroblasts (referred to as Melanocytes and Fibroblasts, respectively) were purchased from Life Technologies (Grand Island, NY). Melanocytes are cultured in Medium 254 supplemented with HMGS (Human Melanocyte Growth Supplement) and Fibroblasts are cultured in FibroLife Medium (Lifeline Cell Technology; Frederick, M.D.). These cells are maintained in presence of 37°C and 5% CO_2.

For the treatment, Melanocytes or Fibroblasts were gently mixed with 200 μg/mL and 400μg/mL miR-302 precursors (dissolved in Mello's F5 formulation) and then seeded in each well of 6-well plates (approx. 30-40 X10^4 cells per well). Cells were also treated with corresponding volumes of F5 alone as solvent control. Cells were removed and collected using trypsin following five (5) days of treatment and the number of viable cells was determined using a hemacytometer.

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RESULTS

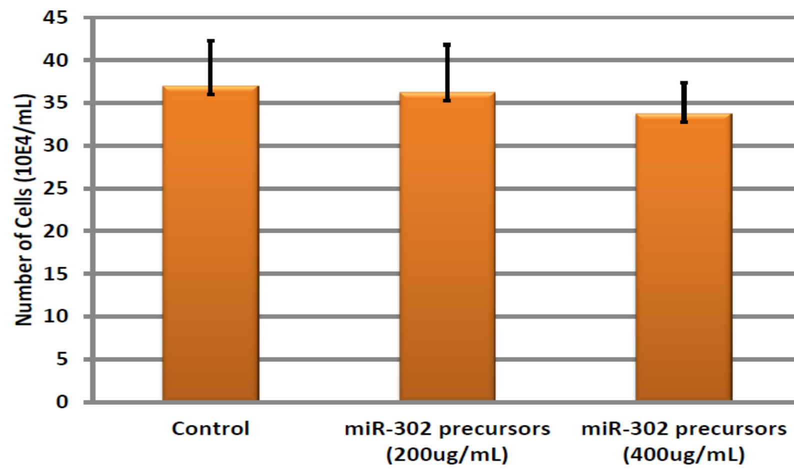

Fig. 1A Effect of F5-formulated miR-302 precursors on Melanocyte cell viability. Cells were treated with 200µg/mL and 400µg/mL miR-302 precursors dissolved in F5. Data are presented as means±SEM ($p<0.05$).

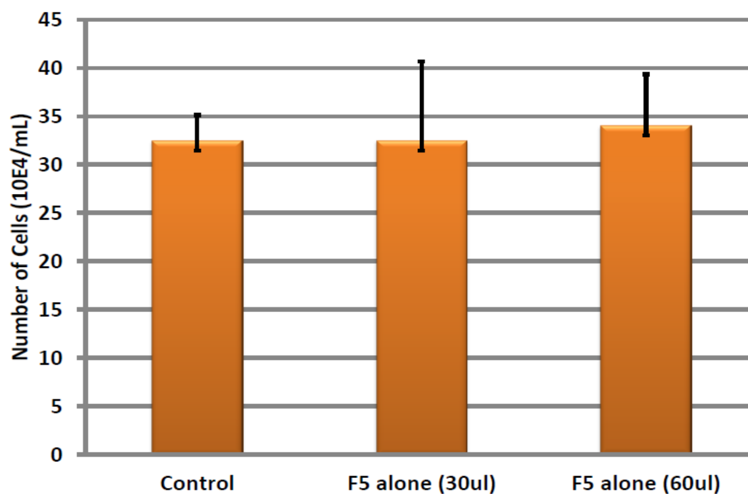

Fig. 1B Effect of F5 on Melanocyte cell viability. Cells were treated with 30µL and 60µL F5, which correspond to the amounts of F5 containing 200µg/mL and 400µg/mL miR-302 precursors, respectively, used in the experiment described in Fig. 1A. Note that this treatment also did not cause detectable reductions in the number of viable cells, as expected. Data are presented as means±SEM ($p<0.05$).

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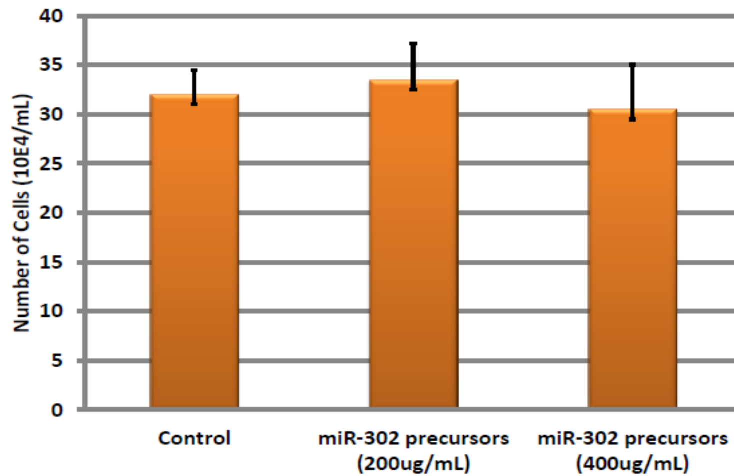

Fig. 2A Effect of F5-formulated miR-302 precursors on Melanocyte cell viability. This figure summarizes data from an experiment repeating the one described in Fig. 1A. Once again, cells were treated with 200µg/mL and 400µg/mL miR-302 precursors dissolved in F5. Data are presented as means±SEM ($p<0.05$).

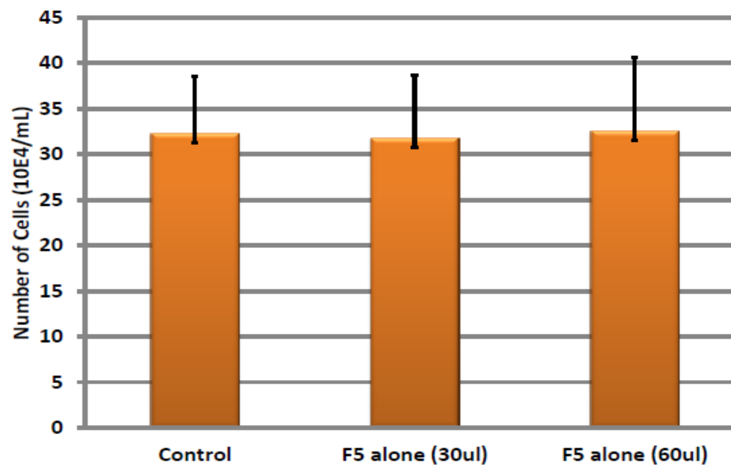

Fig. 2B Effect of F5 on Melanocyte cell viability. This figure summarizes data from an experiment repeating the one described in Fig. 1B. Once again, cells were treated with 30µL and 60µL F5, which correspond to the amounts of F5 containing 200µg/mL and 400µg/mL miR-302 precursors, respectively, used in the experiment described in Fig. 2A. Note that this treatment also did not cause detectable reductions in the number of viable cells, as expected. Data are presented as means±SEM ($p<0.05$).

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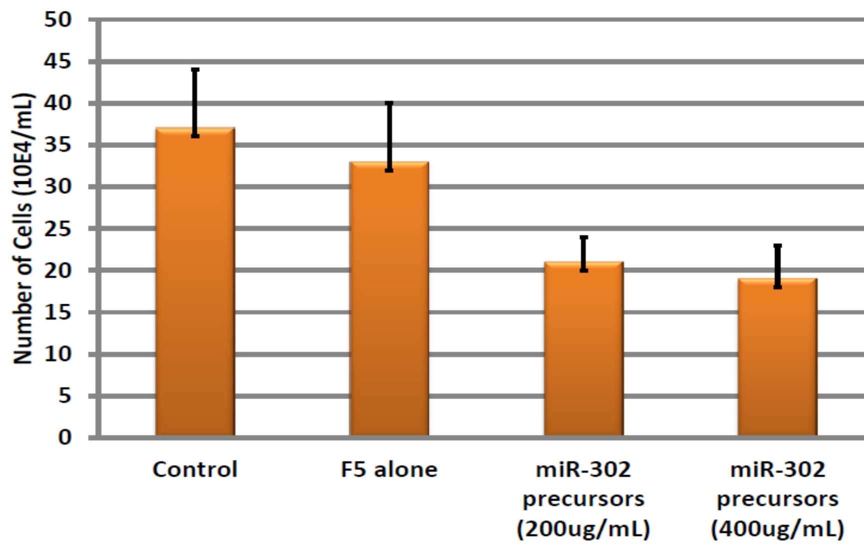

Fig. 3A Effect of F5-formulated miR-302 precursors on Fibroblast cell viability. Cells were treated with 200µg/mL and 400µg/mL miR-302 precursors dissolved in F5. Data are presented as means±SEM ($p<0.05$).

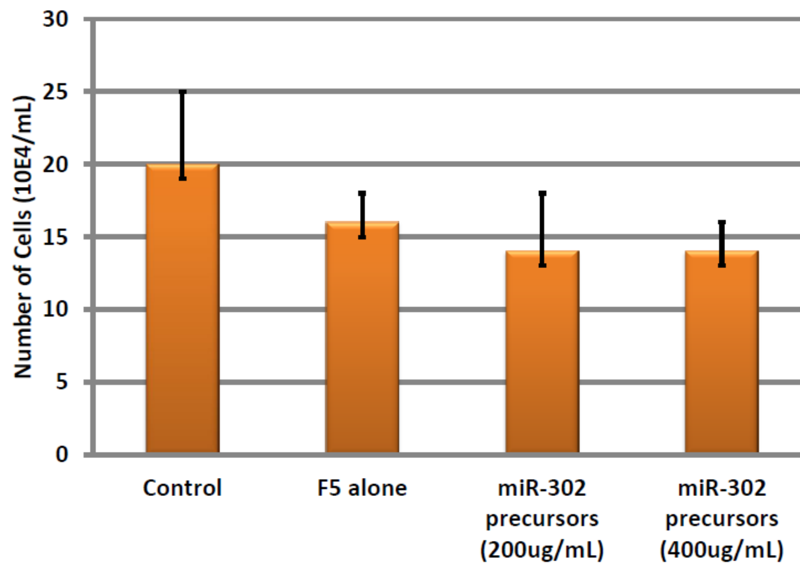

Fig. 3B Effect of F5-formulated miR-302 precursors on Fibroblast cell viability. This figure summarizes data from an experiment repeating the one described in Fig. 3A. Once again, cells were treated with 200µg/mL and 400µg/mL miR-302 precursors dissolved in F5. Data are presented as means±SEM ($p<0.05$).

CONCLUSION

In this study, we assessed the cytotoxic effects of F5-formulated miR-302 precursors (200µg/mL and 400µg/mL) on human melanocytes, fibroblasts and keratinocytes. As shown in Figs 1A, 2A, 1B and 2B, our data clearly showed that treatment with both F5-formulated miR-302 precursors as well as F5 alone did not significantly reduce the number of viable cells. On the other hand, as shown in Figs 3A and 3B, treatment with F5 alone and F5-formulated miR-302 precursors resulted in significantly more noticeable reduction in the viability of fibroblasts. In summary, our study showed that miR-302 precursors are non-toxic to human melanocytes but moderately toxic to human fibroblasts.

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