Effect of rapamycin (serolimus) on *in vitro* cultured human keratinocytes

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Introduction

Novel approaches to reduce the gap between clinical studies and experimental basic research of skin physiology are needed. It is known that rapamycin inhibits TOR (target of rapamycin) signaling, a molecule that stimulates cell growth by regulating protein synthesis [1]. While rapamycin has been extensively studied and used in organ transplantation [2], extensive studies of the effect on human skin cells have not been performed. In this study, we observed the effect of rapamycin on previously frozen human skin keratinocytes.

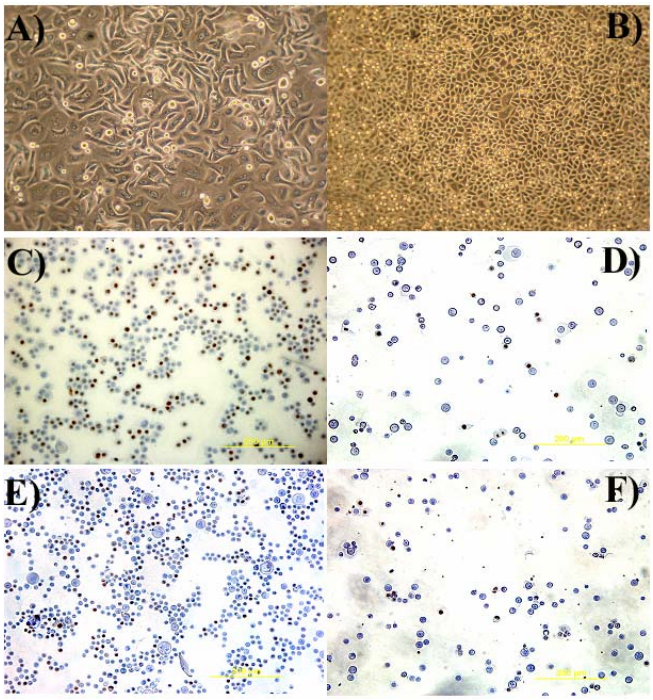

Figure 1: Top row. Effect of rapamycin on human keratinocytes obtained from breast biopsies at day 10 of culture. Left, keratinocytes in regular medium, right, in rapamycin medium. Second row, p63 expression in "popped cells" from the regular medium keratinocytes at the beginning (left) and end of the culture. Third row, same as second row for rapamycin treated cells.

Materials and Methods

Keratinocytes were seeded from a previously frozen culture at passage zero from full thickness human skin explants from discarded breast reductions [3]. The culture medium used was EpiLife® (Cascade Biologics, Portland, OR) in low calcium medium. Rapamycin treated cells were cultured with the same medium with rapamycin at 2nM concentration. Cultured cells were started the same day and seeded in separate flasks. After reaching confluency at day 5 of plating, one flask was changed to rapamycin medium and the other was maintained in rapamycin free medium. The cultures do not contact inhibit but continue growing, releasing cells ("popping" cells) into the overlying medium from the confluent monolayer. Cells released to the medium were studied for expression of p63. p63 is a basal cell marker which, in keratinocytes, is presumed to maintain epithelial cell proliferation.

Results

Figure 1 shows the evolution of the cultured cells. Initial extraction of the cells produced a keratinocyte culture that evolved to form a keratinocyte monolayer. At day 10 of the culture, breast cells cultured with regular medium showed a monolayer of elongated cells (Figure 1A), but the rapamycin treated one (5 days on rapamycin medium, Figure 1B) maintained the monolayer almost intact. It was also observed that rapamycin had the effect of making the cells smaller and the monolayer more compact (Figure 1B). During the time of the culture both flasks released cells to the overlying medium. Analysis of these cells indicated that p63 was highly expressed in the regular medium treated cells at the beginning of the culture (Figure 1C) and decreased over time, 9 day later (Figure 1D). At the beginning of the culture, it was observed a substantial decrease of p63 with rapamycin (Figure 1E) and these low levels of expression are maintained over time, 9 days later (Figure 1F).

Discussion and Conclusions

Rapamycin appears to extend the useful life of the monolayers of keratinocytes while maintaining the epithelial characteristics of the "popped" cells. While cultured cells lose p63 expression over time, rapamycin accelerates this phenomenon from the beginning. Thus the effect of rapamycin is to maintain low P63 expression over time on frozen cells with the effect of "slowing" cell proliferation. This finding may be of interest in tissue engineering or clinical applications where previously frozen cells are used. Additional studies of primary skin keratinocytes in different conditions with rapamycin are currently underway to better assess short and long-term rapamycin effects.

References

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