Stem Cell Reports
Article

ISSCR

OPEN ACCESS

Identification of Drugs that Regulate Dermal Stem Cells and Enhance Skin Repair

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http://dx.doi.org/10.1016/j.stemcr.2015.12.002

SUMMARY

Here, we asked whether we could identify pharmacological agents that enhance endogenous stem cell function to promote skin repair, focusing on skin-derived precursors (SKPs), a dermal precursor cell population. Libraries of compounds already used in humans were screened for their ability to enhance the self-renewal of human and rodent SKPs. We identified and validated five such compounds, and showed that two of them, alprostadil and trimebutine maleate, enhanced the repair of full thickness skin wounds in middle-aged mice. Moreover, SKPs isolated from drug-treated skin displayed long-term increases in self-renewal when cultured in basal growth medium without drugs. Both alprostadil and trimebutine maleate likely mediated increases in SKP self-renewal by moderate hyperactivation of the MEK-ERK pathway. These findings identify candidates for potential clinical use in human skin repair, and provide support for the idea that pharmacological activation of endogenous tissue precursors represents a viable therapeutic strategy.

INTRODUCTION

Advances in adult tissue stem cell biology have led to the idea that pharmacological activation of resident stem cells might represent a therapeutic strategy for tissue repair (Miller and Kaplan, 2012). Indeed, pharmacological candidates that regulate tissue stem cells have been identified including, for example, metformin for neural precursors (Wang et al., 2012; Dadwal et al., 2015) and StemRegenin 1 for primary human hematopoietic stem cells (Boitano et al., 2010). Here, we asked whether this is a viable strategy for skin repair. Skin is a complex tissue with many endogenous tissue stem cells. These include epidermal stem cells (Hsu et al., 2014) and a population of dermal stem cells called skin-derived precursors (SKPs) (Toma et al., 2001, 2005). Cultured SKPs clonally generate mesenchymal progeny like dermal fibroblasts and adipocytes, and peripheral neural progeny like Schwann cells, consistent with the finding that they derive from both neural crest and mesodermal origins (Fernandes et al., 2004; McKenzie et al., 2006; Jinno et al., 2010; Krause et al., 2014), like the dermis itself. With regard to their in vivo function, cultured SKPs can clonally reconstitute the dermis and induce hair follicle morphogenesis (Biernaskie et al., 2009), suggesting key roles for the endogenous precursors in dermal maintenance and hair follicle biology.

Here, we have tested the idea that increasing the number and/or self-renewal of endogenous SKPs would enhance skin repair. To do so, we screened libraries of compounds that are used clinically in humans, looking for drugs that enhance SKP self-renewal. We identified two compounds, alprostadil and trimebutine maleate (TM), that increased SKP self-renewal, likely by activating the MEK-ERK pathway. Both compounds enhanced wound healing when applied topically. These findings provide proof of principle for the idea that compounds that regulate SKPs in culture have therapeutic efficacy in vivo, and identify potential drug candidates that can be repositioned for use in humans.

RESULTS

Screens to Identify Compounds that Enhance Human and Rodent SKP Self-Renewal and Proliferation

We performed high-throughput proliferation screens using primary human foreskin SKPs and neonatal rat dorsal SKPs grown as spheres in serum-free growth medium containing

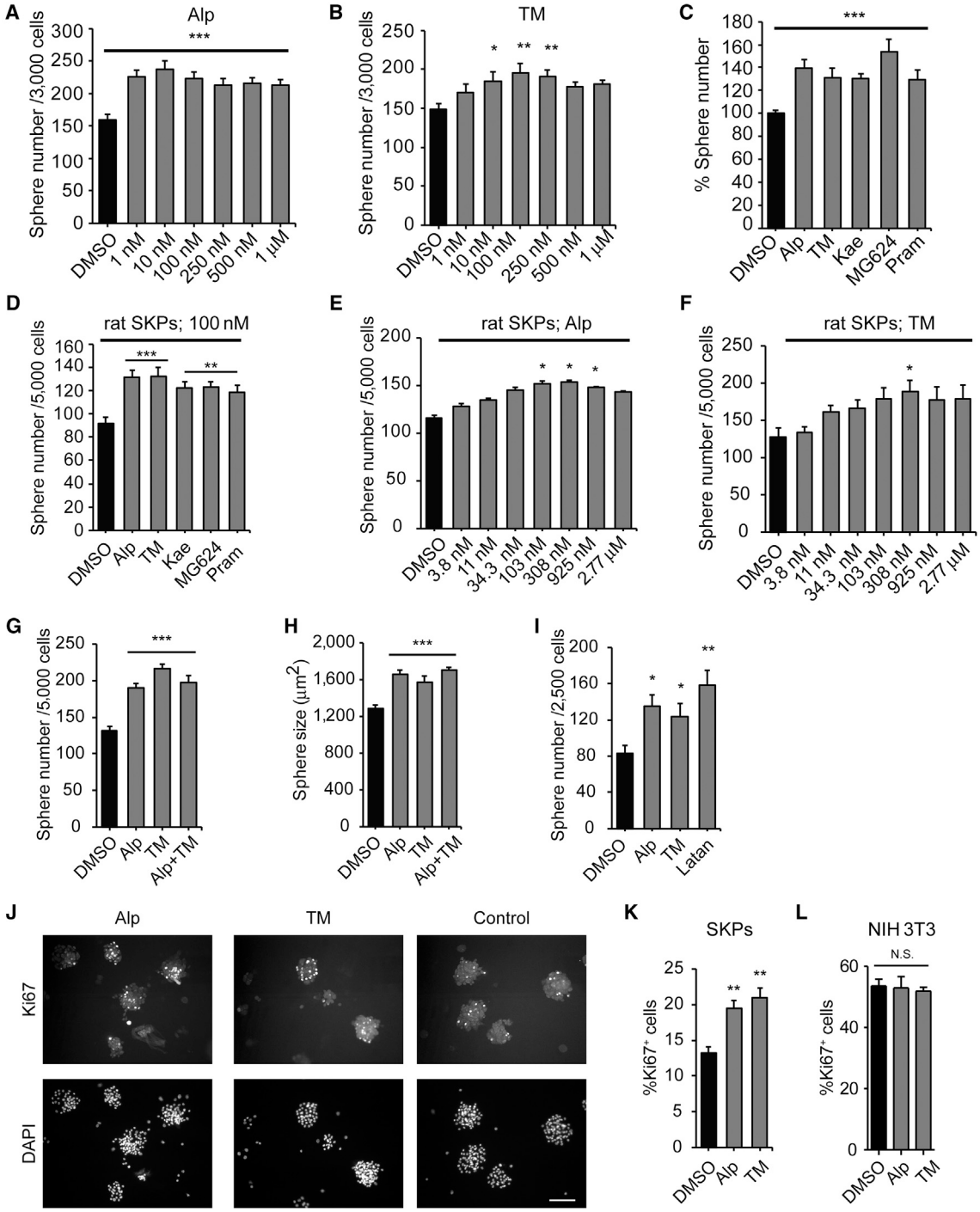

Figure 1. Identification of Compounds that Enhance Self-Renewal and Proliferation of Cultured SKPs

(A–C) Number of SKP spheres generated from secondary human SKPs grown for 7 days in varying concentrations of alprostadil (Alp) (A) or TM (B) or in 100 nM alprostadil, TM, kaempferol (Kae), MG-624, or pramoxine (Pram) (C). In (C) numbers are expressed relative to DMSO alone.

(D–F) Number of SKP spheres generated from secondary neonatal rat SKPs grown for 7 days in 100 nM of each of the five drugs (D), or in varying concentrations of alprostadil (E) or TM (F).

(G and H) Number (G) and size (H) of rat SKP spheres generated over 7 days in 100 nM alprostadil, TM, or both.

(I) Number of rat SKP spheres generated in 14-day clonal methylcellulose assays with 100 nM alprostadil, TM, or latanoprost (Latan).

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