Pachyonychia Congenita Project

15 March 2005

Use of Articles in the Pachyonychia Congenita Bibliography

The articles in the PC Bibliography may be restricted by copyright laws. These have been made available to you by PC Project for the exclusive use in teaching, scholarship or research regarding Pachyonychia Congenita.

To the best of our understanding, in supplying this material to you we have followed the guidelines of Sec 107 regarding fair use of copyright materials. That section reads as follows:

> Sec. 107. - Limitations on exclusive rights: Fair use
> Notwithstanding the provisions of sections 106 and 106A, the fair use of a copyrighted work, including such use by reproduction in copies or phonorecords or by any other means specified by that section, for purposes such as criticism, comment, news reporting, **teaching (including multiple copies for classroom use), scholarship, or research**, is not an infringement of copyright. In determining whether the use made of a work in any particular case is a fair use the factors to be considered shall include - (1) the purpose and character of the use, including whether such use is of a commercial nature or is for nonprofit educational purposes; (2) the nature of the copyrighted work; (3) the amount and substantiality of the portion used in relation to the copyrighted work as a whole; and (4) the effect of the use upon the potential market for or value of the copyrighted work. The fact that a work is unpublished shall not itself bar a finding of fair use if such finding is made upon consideration of all the above factors.

We hope that making available the relevant information on Pachyonychia Congenita will be a means of furthering research to find effective therapies and a cure for PC.

2386 East Heritage Way, Suite B, Salt Lake City, Utah 84109 USA
Phone +1-877-628-7300 • Email—Info@pachyonychia.org
www.pachyonychia.org

Correspondence

The chemical chaperone trimethylamine *N*-oxide ameliorates the effects of mutant keratins in cultured cells

DOI: 10.1111/j.1365-2133.2008.08596.x

Sir, Mutations in keratin genes are the cause of at least 19 distinct disorders, many of which affect the skin and its appendages.[1] The clinical symptoms vary according to the expression pattern of the mutated keratin but typically involve cell fragility and hyperkeratosis. Although keratin disorders are rarely life threatening, they can be painful and debilitating and most treatments are limited to pain relief and prevention of infection. The observation that many keratin mutations are associated with cytoplasmic keratin aggregates led us to hypothesize that keratin disorders are protein-folding disorders and that chemical chaperones, which promote normal

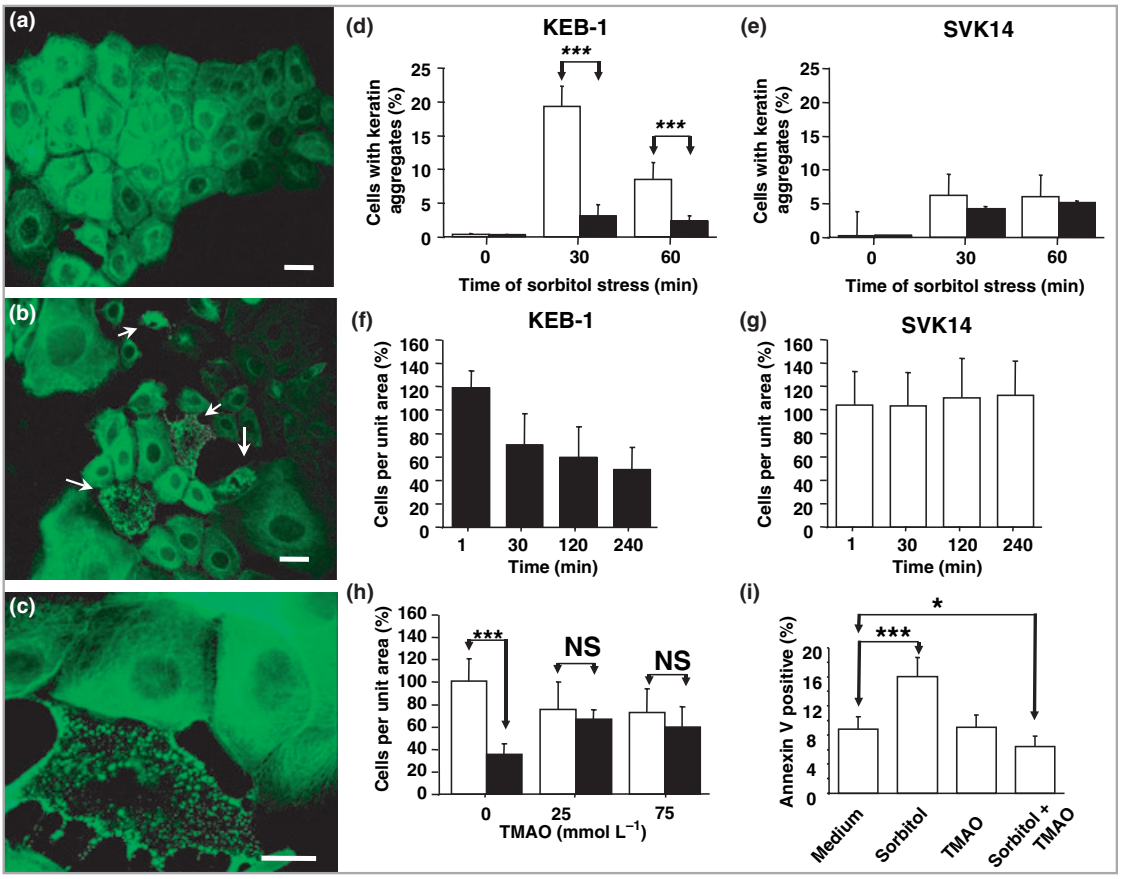

Fig 1. KEB-1 cells stained with the pankeratin antibody LP34 under normal growth conditions (a) or following exposure to 300 mmol L^{-1} sorbitol (b and c). Scale bars = 25 μm. The effects of TMAO on hyperosmotic stress-induced keratin aggregate formation in KEB-1 and SVK14 are shown in (d) and (e), respectively. Normally growing cells (open bars) or cells that had been incubated with 75 mmol L^{-1} TMAO for 24 h (filled bars) were exposed to 300 mmol L^{-1} sorbitol for 0, 30 or 60 min. Cells were fixed in methanol/acetone (50/50 v/v) and stained for keratins. The percentage of cells containing keratin aggregates was determined by counting at least 1000 cells per experimental condition. KEB-1 (f) or SVK14 (g) cells were exposed to hyperosmotic stress for various times and the number of cells remaining attached to the culture dish determined. The effect of TMAO on KEB-1 cells was determined by treating cells with 25 mmol L^{-1} or 75 mmol L^{-1} TMAO for 24 h followed by hyperosmotic stress (h). Open bars indicate no stress, filled bars indicate exposure to 300 mmol L^{-1} sorbitol for 2 h. (i) The effects of TMAO on annexin V positive KEB-1 cells grown under normal culture conditions and following 2 h exposure to 300 mmol L^{-1} sorbitol. Results are expressed as means and standard deviations. Statistical differences were determined using the Student's t-test (*$P < 0.05$; ***$P < 0.001$).

protein folding, will reduce the cellular effects of the mutant keratins.[2]

To test this hypothesis we examined the ability of the chemical chaperone trimethylamine N-oxide (TMAO) to reduce the effects of hyperosmotic stress in cells expressing mutant keratins.

We first examined the effect of hyperosmotic stress on a cell line derived from a patient with epidermolysis bullosa simplex (KEB-1; K5:Glu475Gly).[3] Incubation of KEB-1 cells with medium containing 300 mmol L^{-1} sorbitol for 30 min led to collapse of the keratin cytoskeleton and the formation of cytoplasmic keratin aggregates in more than 20% of the cells. In contrast, keratin aggregates formed fewer than 5% of controls cells (SVK14 cell line) following similar treatment. Pretreatment of KEB-1 cells with TMAO reduced the number of cells with keratin aggregates to control levels (Fig. 1). A decrease in the number of keratin aggregate-containing cells was noted after prolonged exposure (> 30 min) of KEB-1 cells to hyperosmotic conditions. This could indicate that cells containing aggregates were lost from the culture dish or that the keratinocytes were re-establishing their keratin cytoskeleton. To distinguish between these possibilities, KEB-1 and SVK14 cells were incubated with sorbitol for various times and the number of cells remaining attached to the plate determined. There was a time-dependent decrease in the number of KEB-1 cells indicating that cells containing keratin aggregates were preferentially lost from the culture dish. No effect on cell number was observed in control cells. Pretreatment with TMAO protected KEB-1 cells from hyperosmotic-induced cell loss (Fig. 1).

To investigate if apoptosis played a role in KEB-1 cell loss, cells were stained with annexin V, which is an early marker of apoptosis. Approximately 8% of KEB-1 cells growing in normal culture medium expressed annexin V on the cell membrane. This level of apoptotic cells is similar to that reported in HaCaT and HeLa cells.[4] Exposure of KEB-1 cells to sorbitol led to a twofold increase in the number of annexin V positive cells. Treatment of cells with TMAO prior to hyperosmotic stress reduced the number of positive cells to levels that were not significantly different from controls (Fig. 1).

To determine if hyperosmotic stress-induced keratin aggregation is a general feature of mutant keratins, cells expressing a different mutant keratin were subjected to similar osmotic stress. MCF-7 cells were transfected with an expression vector

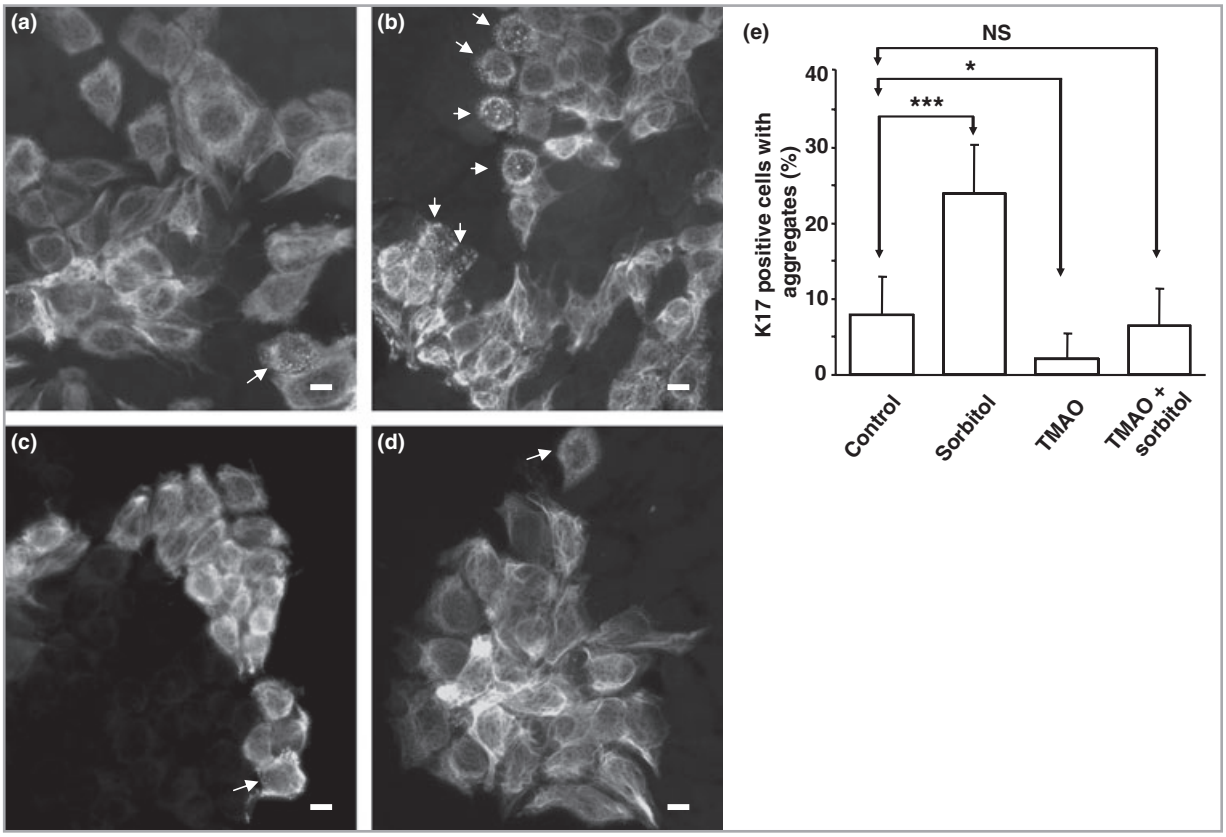

Fig 2. Representative photomicrographs of MCF-7 cells stained for K17:N92S expression following incubation in normal growth medium (a), 5 min in medium containing 300 mmol L^{-1} sorbitol (b), 24 h in medium containing 25 mmol L^{-1} TMAO (c) or 24 h in medium containing 25 mmol L^{-1} TMAO followed by 5 min exposure to 300 mmol L^{-1} sorbitol (d). Scale bars = 10 μm. Arrows indicate cells containing keratin aggregates. The percentage of K17 positive cells containing keratin aggregates is shown in (e). Results are expressed as means and standard deviations. Statistical differences were determined using the Student's *t*-test (*$P < 0{\cdot}05$; ***$P < 0{\cdot}001$).

containing the coding sequence for K17:Asp92Ser, a common keratin mutation causing pachyonychia congenita type 2.[5] MCF-7 cells do not express K17 and thus exogenous expression can be detected directly by immunostaining. Under normal growth conditions K17:Asp92Ser formed filaments in most cells although keratin aggregates were observed in a few cells. Exposure of cells to sorbitol resulted in a threefold increase in the number of cells containing keratin aggregates. Pretreatment of cells with TMAO significantly reduced the percentage of cells with keratin aggregates under normal incubation conditions and following exposure to sorbitol (Fig. 2).

Our results demonstrate that cells expressing mutant keratins have an increased sensitivity to hyperosmotic stress and this sensitivity is reduced by the chemical chaperone TMAO. The molecular mechanism by which mammalian cells sense hyperosmotic stress is not clear but it has been proposed that membrane perturbations lead to clustering of EGF receptors and activation of downstream signalling pathways.[6,7] Our results raise the possibility that the keratin cytoskeleton may play a role in regulating the activation of these pathways. TMAO is a naturally occurring osmolyte found at high levels in many marine fish where it counteracts the protein-destabilizing effects of urea and hydrostatic pressure.[8] There is evidence that TMAO is effective only in situations where the perturbation in protein folding is caused by other osmolytes.[8] Keratinocytes expressing mutant keratins also have altered responses to hypo-osmotic stress and changes in temperature.[3,9] It remains to be established if the effects of these stressors can be reduced by TMAO or if other chemical chaperones are as effective as TMAO. Nevertheless, our observations provide evidence that, at least in some circumstances, chemical chaperones are effective at reducing the cellular effects of mutant keratins and might provide a pharmacological approach to treating keratin disorders.

Acknowledgments

This work was supported in part by the University of California, Irvine Undergraduate Research Opportunity Program.

*Department of Dermatology, University of California Irvine, C340 Medical Sciences, Irvine, CA 92697, U.S.A.
†Centre for Cutaneous Research, Barts and The London, Queen Mary's School of Medicine and Dentistry, London, U.K.
Correspondence: Elizabeth L. Rugg.
E-mail: erugg@uci.edu

D. Lee*
D. Santos*
H. Al-Rawi†
A.M. McNeill*
E.L. Rugg*†

References

1 Rugg EL, Leigh IM. The keratins and their disorders. *Am J Med Genet C Semin Med Genet* 2004; **131C**:4–11.
2 Bernier V, Lagace M, Bichet DG *et al.* Pharmacological chaperones: potential treatment for conformational diseases. *Trends Endocrinol Metab* 2004; **15**:222–8.
3 Morley SM, Dundas SR, James JL *et al.* Temperature sensitivity of the keratin cytoskeleton and delayed spreading of keratinocyte lines derived from EBS patients. *J Cell Sci* 1995; **108**:3463–71.
4 Daehn IS, Varelias A, Rayner TE. Sodium butyrate induced keratinocyte apoptosis. *Apoptosis* 2006; **11**:1379–90.
5 Smith FJ, Corden LD, Rugg EL *et al.* Missense mutations in keratin 17 cause either pachyonychia congenita type 2 or a phenotype resembling steatocystoma multiplex. *J Invest Dermatol* 1997; **108**:220–3.
6 de Nadal E, Alepuz PM, Posas F. Dealing with osmostress through MAP kinase activation. *EMBO Rep* 2002; **3**:735–40.
7 Rosette C, Karin M. Ultraviolet light and osmotic stress: activation of the JNK cascade through multiple growth factor and cytokine receptors. *Science* 1996; **274**:1194–7.
8 Yancey PH. Organic osmolytes as compatible, metabolic and counteracting cytoprotectants in high osmolarity and other stresses. *J Exp Biol* 2005; **208**:2819–30.
9 D'Alessandro M, Russell D, Morley SM *et al.* Keratin mutations of epidermolysis bullosa simplex alter the kinetics of stress response to osmotic shock. *J Cell Sci* 2002; **115**:4341–51.

Key words: *chemical chaperone, genodermatoses, hyperosmotic stress, keratin*

Conflicts of interest: none declared.