A HUMAN CELLULAR MODEL OF DARIER DISEASE DEFINES NEW POTENTIAL TREATMENT STRATEGIES

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BACKGROUND

Darier disease (DD) is a rare autosomal dominant skin blistering disorder caused by mutations in the endoplasmic reticulum (ER) calcium pump, SERCA2. Patients with DD suffer from painful epidermal erosions, recurrent skin infections, and comorbid psychiatric disease, yet it has no FDA-approved therapies. DD biopsies show disruption of cell-cell junctions called desmosomes between keratinocytes, but it is unknown how this occurs.

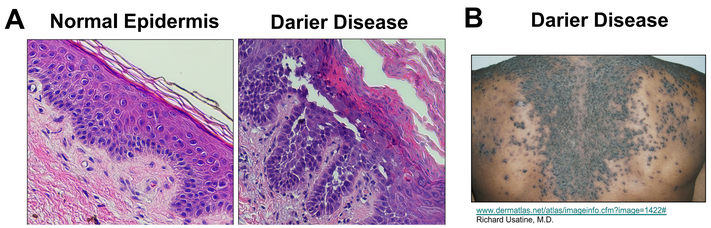

Figure 1: (A) H&E-stained skin biopsy specimens show normal epidermis compared to Darier disease (DD), which demonstrates loss of keratinocyte cohesion (acantholysis) with intra-epidermal blister formation as well as abnormal keratinocyte differentiation (dyskeratosis) with nuclei improperly retained in the cornified layers. (B) Clinical image of affected skin in DD shows numerous eroded papules and plaques with crusting on the upper back.

MODELING DARIER DISEASE

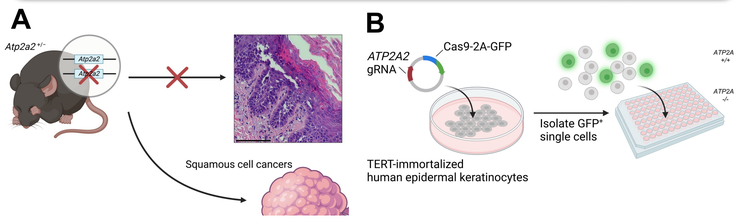

Figure 2: (A) SERCA2 knockout mice did not replicate DD, but instead developed squamous cell carcinomas. (B) TERT-immortalized human epidermal keratinocytes (THEKs) were engineered using CRISPR/Cas9 to knock out (KO) the *ATP2A2* gene that is mutated in DD.

HYPOTHESES

Prior work using this DD model revealed that SERCA2-deficient keratinocytes exhibit excess cytosolic calcium and mis-localization of desmosomal cadherins. We hypothesized that ER calcium leakage impairs cell-cell junctions in DD due to (1) aberrant calcium-induced signaling and/or (2) cadherin mis-folding causing proteotoxic stress in the ER. We further propose that drugs able to mitigate these pathogenic effects of SERCA2 loss may be therapeutic in DD.

CALCIUM SIGNALING AND ER STRESS IN DD

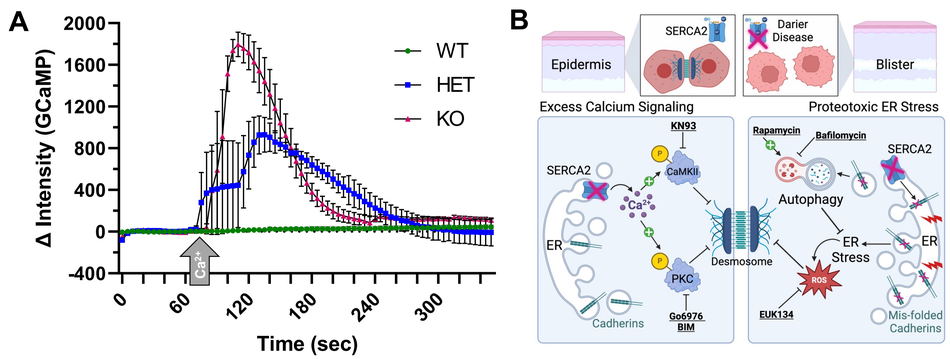

Figure 3: (A) Compared to control cells, human keratinocytes lacking SERCA2 exhibit large cytosolic calcium spikes, which were measured in live cells expressing a green fluorescent calcium sensor (GCaMP). (B) Excess calcium signaling and proteotoxic stress from mis-folded cadherins may both play a role in DD pathogenesis and are potential therapeutic targets.

TESTING NOVEL THERAPIES IN A DD MODEL

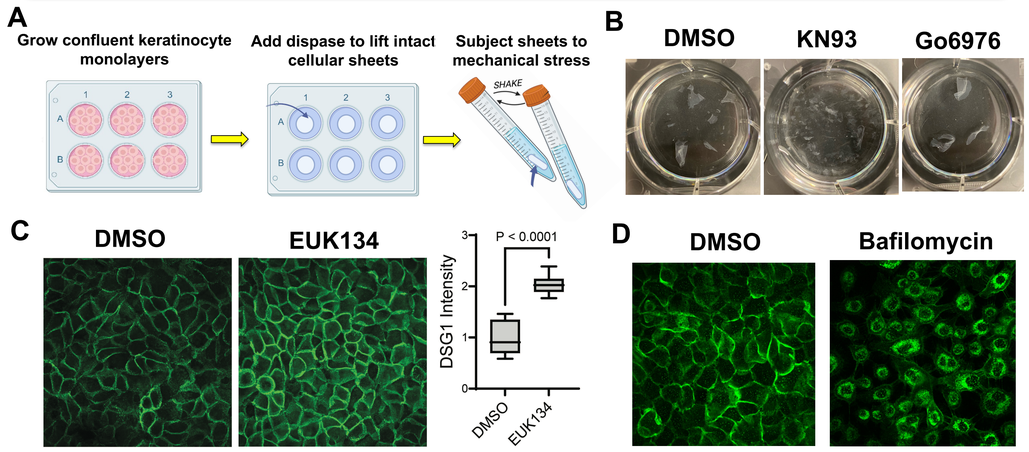

Figure 4: (A) Schematic of a dispase-based mechanical dissociation assay in which intact keratinocyte sheets are subjected to mechanical stress to measure their integrity. (B) *ATP2A2* heterozygous (HET) cells were treated with drugs then were assessed using the assay; while the CaMKII inhibitor KN93 worsened cell-cell-adhesion, PKC-alpha inhibition with Go6976 may improve the cohesion of SERCA2-deficient keratinocytes. (C) Immunofluorescent staining of the desmosomal cadherin DSG1 in HET cells treated with antioxidant EUK 134 showed significantly increased DSG1 intensity indicating that reducing free radicals may improve DD pathology (D) Immunofluorescent staining of the desmosomal cadherin DSG3 in HET cells treated with the autophagy inhibitor bafilomycin showed disruption of cell-cell junctions, indicating that blocking degradation of mis-folded proteins may worsen DD pathology

RESULTS

- CaMKII inhibition weakened cell-cell junctions in SERCA2-deficient cells
- Selective inhibition of PKC-alpha with Go6976 improved desmosome organization in SERCA2-deficient cells
- Go6976 also enhanced keratinocyte sheet integrity early during cell-cell junction assembly, though this effect waned at later time points
- Autophagy activation and inhibition disrupted cell-cell junctions in SERCA2-deficient cells, undermining the potential to target this pathway in DD
- The antioxidant EUK134 significantly enhanced DSG1 intensity in keratinocyte junctions but showed variability in its capacity to strengthen SERCA2-deficient keratinocyte sheet integrity

FUTURE DIRECTIONS

These results suggest that inhibiting PKC-alpha and reducing ROS could represent novel strategies to rescue cell-cell adhesion in DD, but further work is needed to define the ideal timing and dosing of these interventions. In addition, drugs targeting autophagy *weakened* cell-cell junctions in SERCA2-deficient cells, indicating dysregulation of this stress pathway may exacerbate the pathology of DD. In sum, these data demonstrate the potential of our DD model to test and optimize new treatment approaches for this orphan disorder.

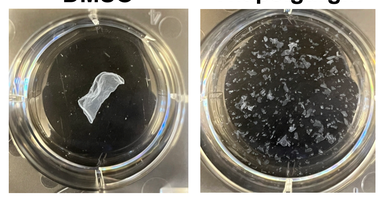

Figure 5: Keratinocyte sheets treated with thapsigargin, a potent chemical inhibitor of SERCA2, showed marked fragmentation compared to DMSO vehicle control. Use of thapsigargin represents an alternative approach to test whether other drugs can rescue cell-cell adhesion in keratinocytes despite dysfunction of SERCA2.

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- **UW Institute for Stem Cell and Regenerative Medicine**
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Plain Language Summary

Darier disease (DD) is a rare genetic skin blistering disorder that is poorly understood and does not have any FDA-approved treatments. We engineered human skin cells (keratinocytes) to replicate the genetics of DD and used these as a model to test new therapies. Two of the treatments tested may be helpful in treating DD, though more rigorous testing is still needed.