Expansion of an HSV-1-Based Gene Therapy Platform to Treat Hailey-Hailey and Darier Diseases

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Background

The topical herpes simplex virus type 1 (HSV-1)-based gene therapy beremagene geperpavec-svdt (B-VEC) has been approved to treat the rare genetic skin blistering disease dystrophic epidermolysis bullosa (DEB). The underlying platform technology is being developed for additional rare skin diseases, including Hailey-Hailey disease (HHD) and Darier disease (DD). HHD and DD are inherited genodermatoses caused by pathogenic variants in the calcium ATPases ATP2C1 and ATP2A2, respectively, for which we are developing KB111 and KB112. Here, we sought to determine if these vectors are capable of transducing clinically relevant keratinocytes both in culture and in wild-type mice in order to express their encoded ATPases with minimal toxicity.

KB111 Transduction Leads to Dose-Dependent ATP2C1 Expression in the Golgi of Keratinocytes Without Cytotoxicity

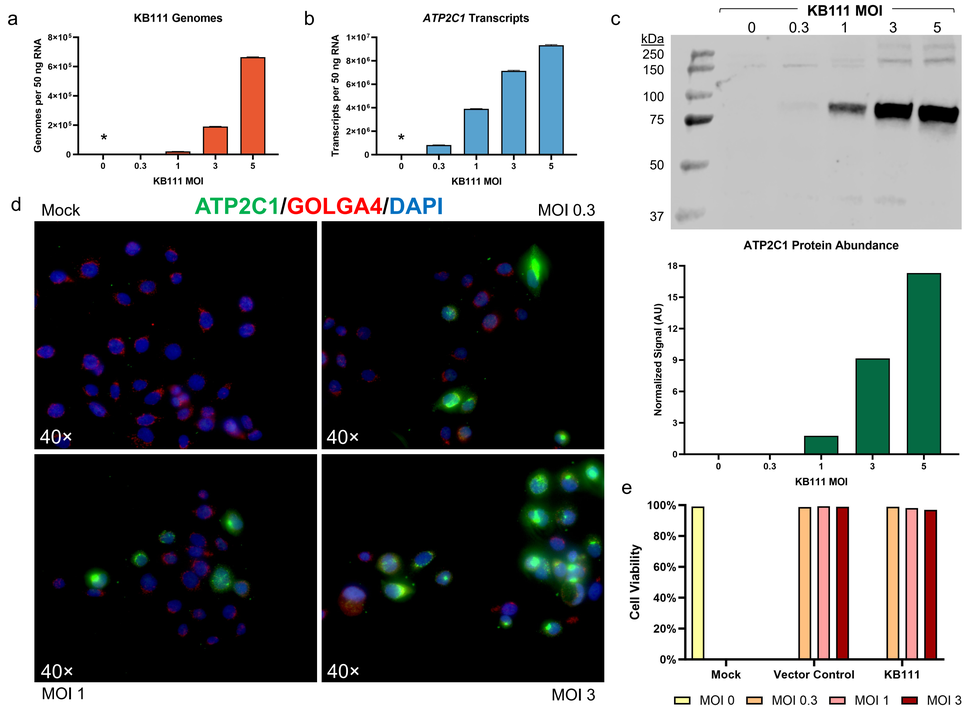

Figure 1. (a) Dose-dependent KB111 genomes via quantitative PCR (qPCR) and (b) *ATP2C1* transgene transcripts via quantitative reverse-transcription PCR (qRT-PCR) in clinically relevant immortalized human keratinocytes after transduction. * = samples < limit of quantification (LOQ) (c, upper) Western blotting (WB) was used to verify the production of full-length ATP2C1 protein and (c, lower) protein quantification confirmed dose-dependent protein expression following transduction at different multiplicities of infection (MOIs). (d) Immunofluorescent (IF) staining revealed proper localization of KB111-expressed ATP2C1 (green) via colocalization with the Golgi marker GOLGA4 (red). DAPI (blue) indicates nuclei staining. (e) Flow cytometry demonstrated cell viability at 48 hours post-transduction, compared to mock-transduced cells or a vector control (same vector backbone as KB111 but lacking *ATP2C1*).

KB111 Functional Correction in an *ATP2C1* Knockdown Cell Model

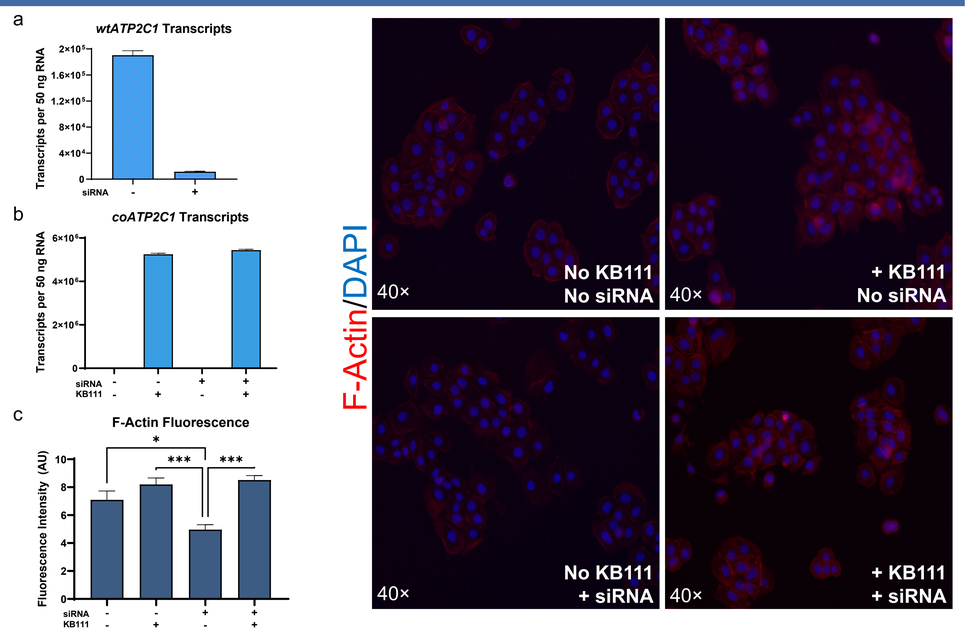

Figure 2. (a) HaCaT cells treated with a small interfering (si) RNA against endogenous ATP2C1 (*wtATP2C1*) demonstrates successful knockdown (KD) via qRT-PCR 24 hours post-transduction. (b) To confirm the ability of KB111 to supplement ATP2C1 expression, codon-optimized (co) *ATP2C1* transcripts were analyzed via qRT-PCR in HaCaT cells following siRNA KD and KB111 transduction. (c) It was previously shown that filamentous (F) actin is reduced in keratinocytes following ATP2C1 KD[1]. F-actin expression was therefore used as a readout for the ability of KB111 to functionally correct ATP2C1 deficiency in siRNA KD keratinocytes. HaCaTs were treated with a combination of siRNA and KB111, fixed in 4% formaldehyde 24 hours after treatment, and stained with the fluorescent F-actin marker phalloidin. (c, left) F-actin fluorescent intensity was quantified across multiple fields and (c, right) representative fluorescent fields are shown. KB111 treatment reversed F-actin loss induced by ATP2C1 KD. * = $p < 0.05$, *** = $p < 0.001$.

KB112 Transduction Leads to Dose-Dependent ATP2A2 Expression in the Endoplasmic Reticulum of Keratinocytes Without Cytotoxicity

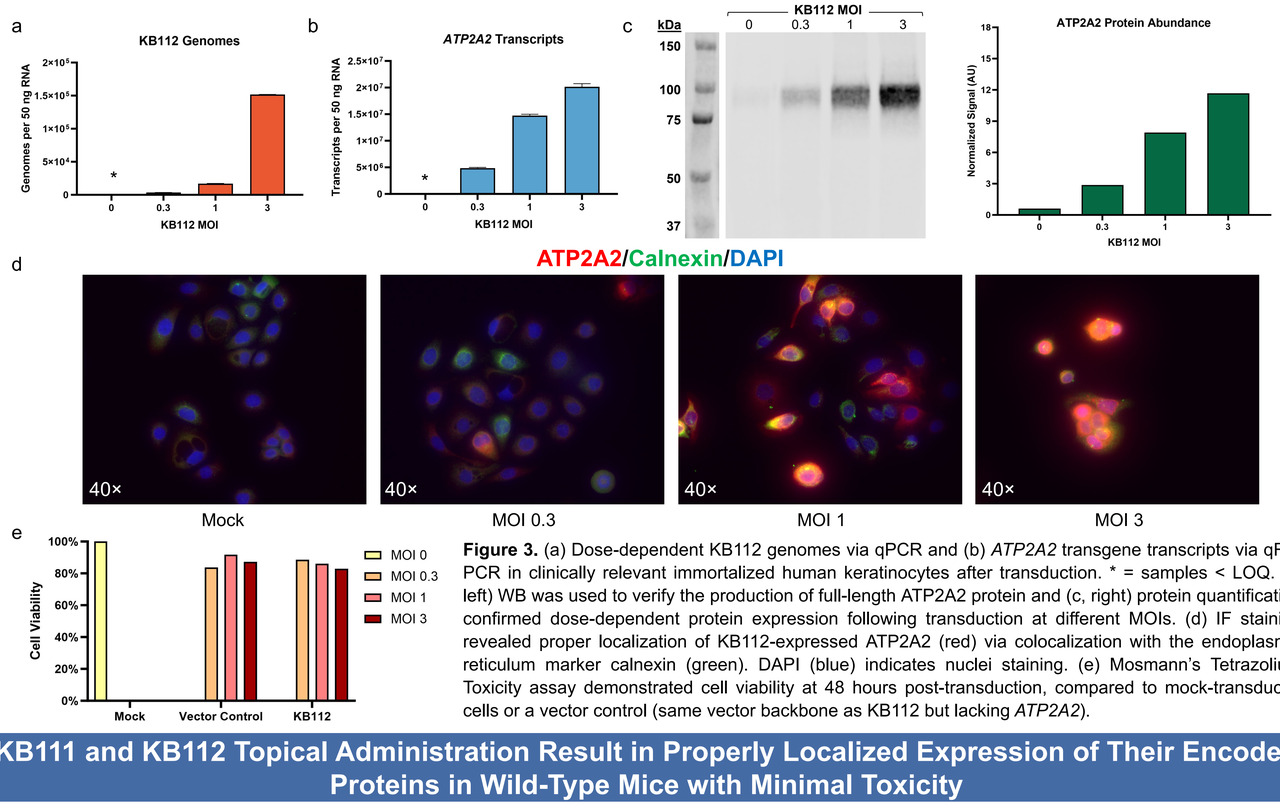

Figure 3. (a) Dose-dependent KB112 genomes via qPCR and (b) *ATP2A2* transgene transcripts via qRT-PCR in clinically relevant immortalized human keratinocytes after transduction. * = samples < LOQ. (c, left) WB was used to verify the production of full-length ATP2A2 protein and (c, right) protein quantification confirmed dose-dependent protein expression following transduction at different MOIs. (d) IF staining revealed proper localization of KB112-expressed ATP2A2 (red) via colocalization with the endoplasmic reticulum marker calnexin (green). DAPI (blue) indicates nuclei staining. (e) Mosmann's Tetrazolium Toxicity assay demonstrated cell viability at 48 hours post-transduction, compared to mock-transduced cells or a vector control (same vector backbone as KB112 but lacking *ATP2A2*).

KB111 and KB112 Topical Administration Result in Properly Localized Expression of Their Encoded Proteins in Wild-Type Mice with Minimal Toxicity

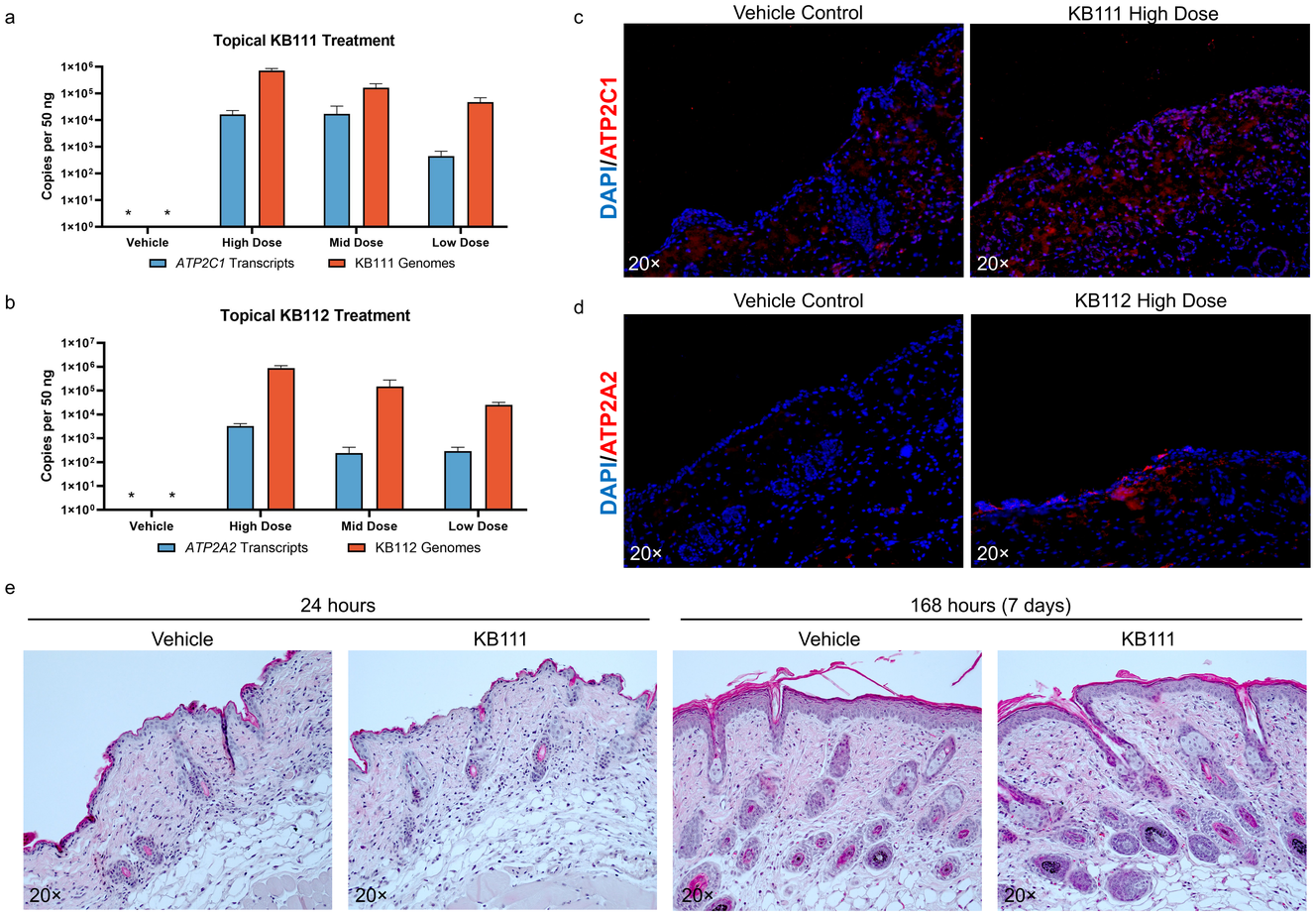

Figure 4. (a,b) Mice were treated topically with high, mid, or low dose (a) KB111 or (b) KB112 mixed with gel to abraded skin of the dorsal thoracic region. Full-thickness skin punch biopsies were collected after 24 hours for qPCR and qRT-PCR analysis of KB111/KB112 genomes and ATP2C1/ATP2A2 transcripts. (c,d) The localization of human (c) ATP2C1 or (d) ATP2A2 was assessed via IF in topically-treated mouse skin. (e) The tolerability of KB111 was evaluated by hematoxylin and eosin staining of skin punch biopsies after a single maximum achievable topical dose. These histological findings are representative of KB112 results.

Conclusions

Both KB111 and KB112 are capable of transducing keratinocytes and expressing their encoded ATPases with minimal toxicity in culture and in murine skin, demonstrating that Krystal's HSV-1-based platform is well-suited for the treatment of both HHD and DD.

Acknowledgements/Disclosures/References

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