

Localization of p63 to basal epithelial cells of the equine (*Equus caballus*) hoof epidermal lamellae.

Hannah Galantino-Homer, University of Pennsylvania School of Veterinary Medicine, Department of Clinical Studies/New Bolton Center, 382 West Street Rd Kennett Square, PA 19348-1692
hghomer@vet.upenn.edu
610-925-6246

Makoto Senoo, University of Pennsylvania School of Veterinary Medicine, Department of Animal Biology, Philadelphia, PA, msenoo@vet.upenn.edu

Tajie Harris, University of Pennsylvania School of Veterinary Medicine, Department of Pathobiology, Philadelphia, PA, tajieh@vet.upenn.edu

David Lorom, University of Pennsylvania School of Veterinary Medicine, Department of Clinical Studies/New Bolton Center dlorom@vet.upenn.edu

The equine hoof is continuously growing at a rate of approximately 1 cm per month. This growth is supported by proliferation of epidermal epithelial cells of the coronary tubules and proximal and distal lamellae, with very low levels of proliferation in the middle lamellar regions of the hoof. In contrast, horses suffering from laminitis, a common and often debilitating condition, often have impaired or abnormal hoof growth and excessive cellular proliferation of mid-lamellar epidermal epithelial cells resulting in the formation of a "lamellar wedge" of epidermal tissue that contributes to the pathology of this disease. Our hypothesis is that equine epidermal lamellar epithelial stem cells maintain proliferative capacity due to the presence of p63, a transcription factor that has been linked to epithelial stem cell proliferative potential in other species. The purpose of this study was to verify and characterize the presence of equine p63 in epidermal lamellar epithelium. Tissues were harvested immediately following euthanasia from five regions of the foot: skin, coronary tubules and adjacent dermis, and epidermal and dermal tissue from the proximal, mid, and distal lamellae which form the interface and anchor between the hoof and underlying pedal bone. Tissues were either snap frozen in liquid nitrogen or fixed in 4% paraformaldehyde, embedded and frozen for cryosectioning. Immunoblots using a mouse monoclonal antibody against human p63 (clone 4A4) and protein extracted with Laemmli sample buffer revealed a single protein band of the appropriate relative molecular mass in all tissues of the foot and adjacent skin. The same p63 antibody was used for immunohistochemistry which demonstrated that p63 localized to the nuclei of basal and parabasal epidermal epithelial cells of the secondary lamellae. Indirect immunofluorescence with this p63 antibody and with a rabbit polyclonal antibody against human cytokeratin-14 (Abcam ab53115) revealed that a subset of cytokeratin-14 positive cells (basal/parabasal epidermal epithelial cells) have nuclear staining for p63. Our data support the presence of an equine p63 homolog in the nuclei of equine epidermal lamellar basal/parabasal epithelial cells. These data also suggest that these cells maintain proliferative potential in all regions of the foot, consistent with the observation that the normally quiescent mid-lamellar epidermal epithelial cells are capable of rapid proliferation upon injury. To our knowledge, this is the first report of an

equine p63 homolog and the first description of putative epithelial stem cells in the equine hoof.

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