Localization and Enumeration of Progenitor Cell Surface Markers and Primordial Keratins in the Laminitic Equine Hoof

Raeanna Simcoe, Dejiang Feng, Jenna Dessauer, Vanessa Pinto, Olivia Abadie, and Mandi J. Lopez

Laboratory for Equine and Comparative Orthopedic Research, Veterinary Clinical Sciences, School Of Veterinary Medicine, Louisiana State University, Baton Rouge, Louisiana, U.S.A.

INTRODUCTION

Equine laminitis is a devastating condition that affects a significant number of horses. The innermost layer of the hoof (stratum internum) is composed of epidermis and dermis with interdigitating primary lamellae, each covered with many secondary lamellae. Detachment of the secondary lamellae results in sinking or rotation of the third phalanx (Fig. 1,2). Alterations in progenitor cells in the basement membrane between lamellae may disrupt normal tissue growth, repair, and function, characteristic of laminitis.

HYPOTHESIS AND OBJECTIVES

The overarching hypothesis was that progenitor cell alterations occur in the secondary lamellae of laminitic hooves.

Objective 1. Compare progenitor cell surface marker [octamer-binding transcription factor (OCT)-4], sex-determining region Y box (SOX)-2, cluster of differentiation (CD)-29, CD-44, and CD-105 as well as primordial keratin (keratin (K14, K15, and K19) mRNA levels in progenitor cells from laminitic and normal hooves with qRT-PCR.

Objective 2. Localize progenitor cells expressing K14 in both normal and laminitic tissue with immunohistochemistry.

METHODS

Laminar tissue from four normal horses and one laminitic horse euthanized for reasons unrelated to this study were used.

Specimens. Laminar tissue and whole hoof sections were collected immediately after euthanization (Fig. 3B,C).

Cell Culture. Laminar tissue was plated for overnight incubation. Progenitor cells were digested and centrifuged to collect the cells. Cells were seeded onto six-well plates selected based on adherence to plastic.

qRT-PCR. Primers were designed for SOX-2, CD-29, CD-44, CD-105, K14, K15, and K19. mRNA was extracted from progenitor cells and reverse transcription was done to acquire cDNA for qRT-PCR. The housekeeping gene was RPL13A.

Immunohistochemistry. Laminar tissue from whole hoof sections was embedded in OCT (Fig. 3D) and 6 micron sections were mounted on slides. Each slide was fixed, permeabilized, blocked, and stained with a primary antibody against CD-29 (BD 610468), CD-44 (VWR BA024A), CD-105 (Abcam 69772), SOX-2 (Abcam 79351), OCT-4 (SC 5279), K14 (Abcam 7800), K15 (Abcam 93944), or K19 (Abcam 7754). After washing, slides were stained with secondary antibody (DyLight 488 goat anti-mouse) and Hoechst dye, then evaluated with fluorescent microscopy.

RESULTS

1. CD-105 and CD-29 mRNA expression is decreased in progenitor cells from laminitic hooves versus normal hooves.

2. SOX-2, CD-44, and K15 mRNA expression is increased in progenitor cells from laminitic hooves versus normal hooves.


DISCUSSION AND CONCLUSIONS

In progenitor cells from laminitic hooves, increased CD-44 and SOX-2 levels suggest hyperproliferation, decreased CD-29 levels are consistent with detachment at the basement membrane, increased K15 indicates lack of differentiation and stratification, and decreased CD-105 indicates lack of vascularization. Together, these results confirm the hypothesis that progenitor cells in the secondary lamellae of laminitic hooves are altered. Based upon these preliminary results, it can be concluded that progenitor cells in laminitic tissue show markers of hyperproliferation and failure to differentiate. This may be the cause of the detachment of the secondary epithelial and dermal lamellae seen clinically with the sinking and rotation of the third phalanx in laminitis. Further investigation is needed to confirm these results on multiple laminitic and normal hooves and to compare the location of the proteins in laminitic and normal tissues in situ. Understanding of the cellular changes occurring in the basement membrane at the junction of the epidermal and dermal lamellae could lead to potential therapy solutions, perhaps using new progenitor cells to promote normal proliferation and differentiation and induce vascularization.

Clinical Relevance. Knowledge of the cellular changes that occur in the basement membrane during laminitis could have application in prevention or cure of laminitis or other diseases of the epidermal-dermal junction, including epidermolysis bullosa and pemphigus foliaceus.

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