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Vitamin D receptor and CD86 expression in the skin of vitamin D-deficient swine

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ABSTRACT

The immunomodulatory role of vitamin D in many diseases is well established. However, the relationship between vitamin D status and skin cancers is unclear. In this study, we examined the effect of vitamin D deficiency and sufficiency on VDR, NF-κB, and CD86 in the epidermis of Yucatan microswine tragi. All of these proteins have known roles in the pathogenesis of cutaneous malignancies such as melanoma and non-melanoma skin cancer. There was weaker and less discrete nuclear staining for VDR and weaker CD86 immunoreactivity with patchy membranous expression in the epidermis of vitamin D-deficient compared to vitamin D-sufficient swine. There was no difference in the immunostaining for NF-κB. Since VDR and CD86 expression are decreased in the setting of melanoma and non-melanoma skin cancers, our findings suggest a potential role of vitamin D-deficiency in the progression of skin malignancies.

Introduction

A role for 25-hydroxyvitamin D and its receptor, VDR, has been implicated in the biology of a number of physiologic and pathophysiologic cutaneous processes including skin aging (Reichrath, 2012), wound healing (Luderer et al., 2013), psoriasis (Hoss et al., 2013), epidermal Langerhans cell functioning (Yasmin et al., 2013), and both melanoma and non-melanoma skin cancer (Nemazannikova et al., 2013). The action of 25-hydroxyvitamin D and VDR in keratinocytes may help protect against non-melanoma skin cancer by regulating cell proliferation and differentiation (Bikle et al., 2013; Lehmann, 2009). With respect to melanocytic lesions, expression of VDR is decreased in melanocytic nevi and even more so in melanoma when compared to normal skin, and is decreased in the skin surrounding these lesions as well (Brozyna et al., 2011). Recently, vitamin D deficiency was demonstrated as an independent predictor of Merkel cell carcinoma tumor size at diagnosis and metastatic recurrence (Samimi et al., 2013).

Nuclear factor kappa B (NF-κB) is a transcription factor that has been implicated in multiple settings of inflammation and neoplasia (Baud and Karin, 2009), including cutaneous pathology (Rao et al., 2011). As a result of the diverse roles that it plays in fundamental cellular pathways, dysregulation of NF-κB and its upstream signaling pathways leads to alterations in growth, proliferation, and inflammatory immune responses (Rao et al., 2011). The function of NF-κB may be modulated intracellularly by CD86, an important cell surface costimulatory protein in the activation of T and B lymphocytes (Njau and Jacob, 2013). CD86 is thought to play a role in cutaneous squamous cell carcinoma and melanoma immunity (Miguel et al., 2012; Njau and Jacob, 2013; Romero-Tlalolini et al., 2013). Its interaction with the NF-κB pathway has further implications in cutaneous pathology that is linked to the growth of non-melanoma skin cancer (Chaudhary et al., 2012), wound healing (Melchionna et al., 2012), atopic dermatitis (Chervet et al., 2010), and hemangiomas and malignant vascular tumors of the skin (Arbiser et al., 2009).

In this investigation, we examined the immunostaining patterns of VDR, NF-κB, and CD86 in normal appearing porcine skin. Utilizing a swine model of vitamin D deficiency, we investigated the impact of vitamin D sufficiency and deficiency on the expression of these molecules.

Methods

Porcine model

Female Yucatan microswine were housed in the Animal Resource Facility of Creighton University, Omaha, NE and cared for according to NIH standards and USDA guidelines. The swine were housed under controlled conditions, 12:12-h light–dark cycle at 20–24 °C, without sunlight to avoid any variation in the serum 25-hydroxyvitamin D levels due to UV exposure. They were fed 1–1.5 lb/swine/day of a high cholesterol diet that was vitamin D deficient (0 IU 25-hydroxyvitamin D/day) or vitamin D-sufficient (2000 IU 25-hydroxyvitamin D/day). The vitamin D-deficient high cholesterol swine diet (Harlan, USA) consisted of 23.9% corn starch, 23.5% sucrose, 19% "vitamin free" casein, 13% maltodextrin, 10% cellulose, 4% soybean oil, and 4% cholesterol. The vitamin D-sufficient high cholesterol diet (Harlan, USA) consisted of 37.2%

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corn (8.5% protein), 23.5% soybean meal (44% protein), 20% chocolate mix, 5% alfalfa, 4% cholesterol, 4% peanut oil, 1.5% sodium cholate, and 1% lard. Venous blood from the ear vein was drawn every 8 weeks to examine serum 25-hydroxyvitamin D levels.

Histology and immunohistochemistry

Skin was obtained from the tragi of female Yucatan microswine post mortem, embedded in paraffin, sectioned (5–6 μm), and mounted on slides. Samples available for assay and analysis belonged to four vitamin D-sufficient animals and two vitamin D-deficient animals. Slides were baked at 60–65 °C for 2 h prior to deparaffinization and then rehydrated. Antigen retrieval was performed by heating slides in PBS with steam for 25 min and tissue was then blocked with BLOXALL endogenous peroxidase blocking solution (Vector Labs). Tissue was incubated with primary antibody including mouse monoclonal anti-VDR (D6) (Santa Cruz Biotech sc-13133; 1:100 in PBS), rabbit monoclonal anti-NF-κB p65 (Abcam ab-131109; 1:400 in PBS), and rabbit monoclonal anti-CD86 (EP1158Y) (Abcam ab-53004; 1:800 in PBS) for one 1 h at room temperature. Negative controls were run in parallel by excluding primary antibody from the incubating solution. Immunohistochemistry was performed with VECTASTAIN Elite ABC kits (Vector Labs) followed by the use of DAB peroxidase substrate kit (Vector Labs). Specimens were counterstained with hematoxylin prior to affixing coverslips with xylene-based mounting media.

Images

Stained sections were visualized in a blinded manner with a Nikon Eclipse Ci light microscope and photographs were taken with a Nikon

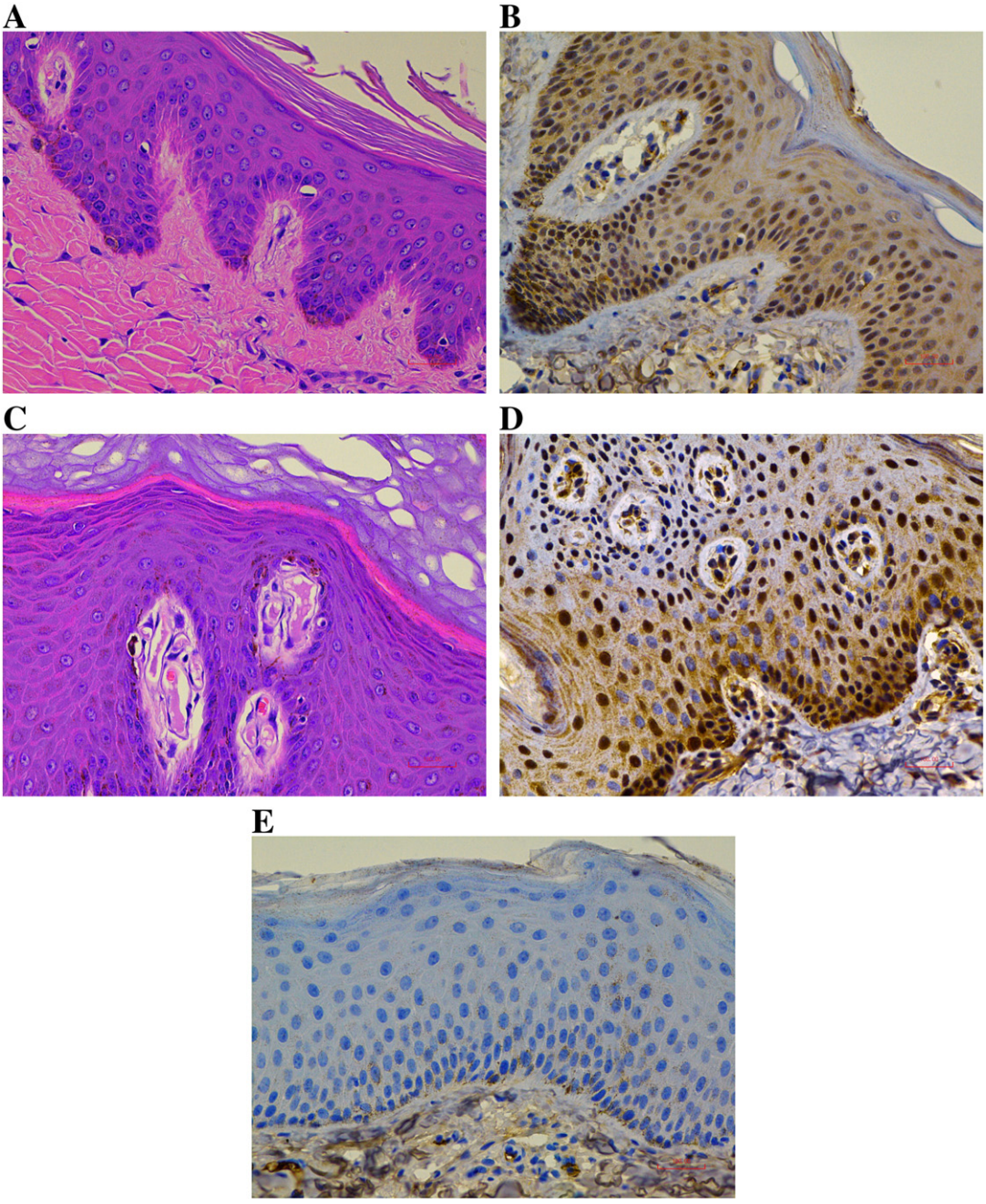

Fig. 1. Hematoxylin and eosin (A, C) and VDR immunohistochemistry (B, D) with negative control (E) from tragi of Yucatan microswine fed vitamin D deficient (A, B) and sufficient (C, D) diets. The H&E and IHC were performed on different tissue sections that were not consecutive and thus have similar but not identical histology. All images were taken at 40× objective.