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Biofilms in Squamous Cell Carcinoma *In Situ*

Herbert B Allen*
Christina Lee Chung
Rina M Allawh
Mary Larijani
Carrie A Cusack

Department of Dermatology, Drexel University College of Medicine, Philadelphia, Pennsylvania, USA

Abstract

We have identified biofilms in squamous cell carcinoma *in situ* (SCCIS) in both sun exposed and non-sun exposed sites in organ transplant recipients (OTRs). The OTRs are at increased risk for skin cancers because of immunosuppression necessary to prevent rejection. The biofilms were discovered by means of pathological staining that has been utilized for recognition of biofilms in many other cutaneous and systemic diseases such as eczema, psoriasis, and Alzheimer's disease. The extracellular polysaccharides, which make up the bulk of the biomass, stain with periodic acid Schiff, and the amyloid that makes up the infrastructure of biofilms stains with Congo red. However, in our specimens, the Congo red was negative; we attribute this to the presence of transthyretin which inhibits the formation of amyloid. Where the majority of the OTRs with SCCIS in the non-sun exposed areas had positive immunostaining for HPV 16 and 18, we considered the possibility that the microbe creating the biofilms was viral. This would be the malignant counterpart to the benign molluscum contagiosum viral lesions in which biofilms are present. If these preliminary observations are repeatable by future observations, SCCIS would join the many other cancers that are associated by viruses.

Keywords

Biofilms; Squamous Cell Carcinoma; *In Situ*

Introduction

We have previously observed that microbial biofilms were important in the pathogenesis of various skin diseases, namely atopic dermatitis, psoriasis, molluscum contagiosum (MC), and others [1-4]. We, and others, have also observed biofilms to be important in non-dermatological diseases such as arthritis, arteriosclerosis, and Alzheimer's disease [5-7]. The biofilms in the cutaneous diseases have been formed by various organisms, such as bacteria, viruses, and yeasts, similar to the situation in nature where the majority of microbes reside in biofilms [8]. Biofilms protect the organisms within from external stresses and, *in vivo*, from the immune system and antibiotics [9]. The protection arises from a coating of polysaccharides made by the microbes; one of the most common coatings is "slime." Another key ingredient of most biofilms is amyloid which is also made by the microbes. The amyloid fibers provide an infrastructure for the polysaccharides; moreover, for pathological examination, the amyloid and polysaccharides are easily stained by Congo red (CR) and periodic acid Schiff (PAS) respectively [1].

Inasmuch as we had identified intracellular biofilms with the MC lesions, we thought it would possibly be appropriate to utilize the same techniques with squamous cell carcinoma *in situ* (SCCIS) in transplant patients because many of these lesions contained high risk HPV strains which could possibly make biofilms [10]. This would then be the malignant counterpart to the benign MC tumors.

Methods

9 specimens from non-sun exposed (NSE) skin from African American (AA) organ transplant recipients (OTR) which had pathologically diagnosed as SCCIS were compared to 11 specimens with SCCIS from sun exposed (SE) skin in Caucasian OTR. Of the 9 specimens from black OTRs, 4 of 6 from the genital area were positive for high risk HPV (16 and 18). The HPV immunostaining was undertaken because of the location of the lesions. All specimens were stained with routine CR and PAS stains. As controls, 20 MC specimens and 20 specimens of healing wounds that had the same staining procedures were examined [3,11]. Four dermatopathologists examined all the tissues. Two additional SCCIS specimens from SE transplant patients were examined with full-field optical coherence tomography (FOCT) microscopy [12].

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*Corresponding author:

Herbert B Allen, MD
112 White Horse Pike
Haddon Heights, New Jersey, USA
Tel: 856 546 5353
Fax: 856 546 8711
E-mail: hba25@drexel.edu

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Results

These specimens had already been diagnosed as SCCIS on routine microscopic examination hematoxylin and eosin staining; this was reconfirmed before beginning the protocol. Markedly positive staining with PAS was noted in the Malpighian zones of SCCIS specimens regardless of whether they were from SE of NSE sites (Figure 1). CR stain in all the specimens was negative regardless of the site (Figure 2). FOCT revealed cells with clear cytoplasm in the Malpighian zone in the same location as the PAS stained cells (Figure 3).

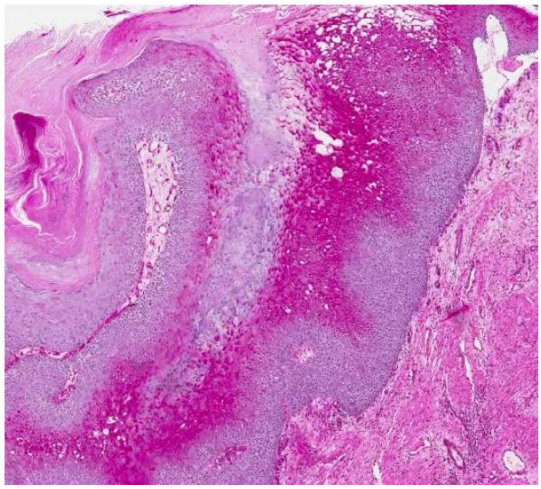

Figure 1: SCCIS stained with PAS-PAS (10X) staining shows marked upper epidermal involvement

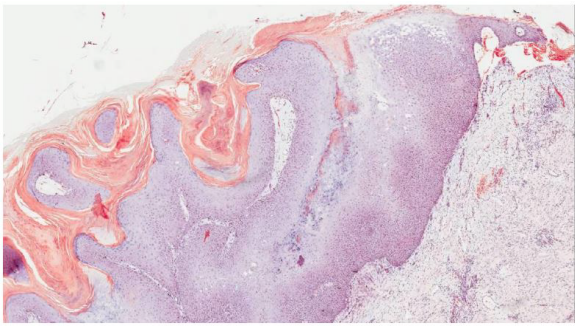

Figure 2: SCCIS stained with Congo red-Congo red (10X) shows lack of staining in upper epidermis

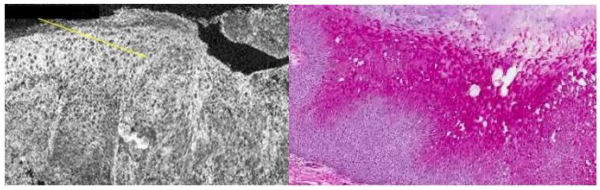

Figure 3: FOCT on left; PAS on right-FOCT, on left, shows washout of bright color from normal to affected area (yellow arrow points to transition zone); PAS 10X, on right, shows positivity in the "washed-out" areas. (FOCT image reprinted with permission from reference 12)

Discussion

The pathological changes in MC and healing wounds with PAS and CR staining have previously been noted [3,11]. The major differences in SCCIS from the controls was the lack of staining with CR (Figure 3). We attribute this to "transthyretin" which is present in cancers and not in benign states [13,14]. Transthyretin has been shown to suppress amyloid formation, and this apparently occurred in this tissue which totally lacks amyloid. It does, however, doubly confirm the fact that these lesions were cancerous.

The PAS staining which was modest in the MC lesions was pronounced in the SCCIS lesions [3]. The difference noted in comparison to the healing wounds was in location of the PAS positivity: in the healing wounds, it was in the sweat duct occlusions *vs* the Malpighian zones in the SCCIS [11]. We interpret these findings as being consistent with biofilms being present in the SCCIS lesions. It is possible in the specimens where the high-risk HPV was identified, that these viruses are the microbes responsible for the biofilms. The other situations where viruses have been shown to make biofilms are in MC3 and HTLV1 [14]. It is postulated in this situation that the virus enters the cell and "hi jacks" the DNA, and the host cell makes the biofilm [14]. We believe the intracellular biofilms are better defined in MC because of the more uniform size of the cells and because of the more ordinary transition of the cells through the epidermis to the stratum corneum. Both of these parameters are disrupted in SCCIS.

In the specimens that were negative for high risk HPV, some strain other than 16 or 18, such as 5 or (others), may ultimately be discovered in these lesions, but this is currently unknown. The presence of the biofilm indicates some microbe is present, however [8]. Viruses have been associated with cancer: HPV, Hepatitis B and C, EB virus, HTLV1, HHV8, and Merkel cell polyoma virus have all been implicated [15,16]. These viruses likely act through different mechanisms, such as incorporation of the viral DNA into the host DNA and through different host factors such as chronic sun damage [17]. How (or if) biofilm is associated with the development of malignancy is yet to be determined.

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