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Activation of Src-family Tyrosine Kinases in hyperproliferative epidermal disorders

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Abstract

Background—Src-family tyrosine kinases (SFKs) are important regulators of keratinocyte growth and differentiation. In a broad range of cell types, persistent activation of SFKs correlates with increased cell proliferation. In this study, we determined if SFK activity is increased in cutaneous neoplasia and psoriasis, common hyperproliferative epidermal disorders.

Methods—Formalin-fixed tissue sections of unremarkable epidermis, psoriasis, actinic keratoses, squamous cell carcinoma in situ (SCIS), and squamous cell carcinoma (SCC) were subjected to immunohistochemical staining for activated SFKs.

Results—All psoriasis specimens displayed significantly greater staining for activated SFKs than sections of unremarkable skin. In the psoriatic biopsies, the degree of epidermal hyperplasia was proportional to the staining for activated SFKs. All actinic keratoses, SCISs, and SCCs exhibited more prominent staining than sections of unremarkable epidermis. Qualitatively, actinic keratoses displayed weaker staining of activated SFKs than the SCC specimens. No discernable difference in activated SFK staining was seen between *in situ* and invasive specimens.

Conclusions—This study demonstrates increased staining of activated SFKs in human biopsy specimens of psoriasis and cutaneous neoplasia. These data provide direct evidence for increased activation of SFKs in the pathogenesis of hyperproliferative epidermal disorders.

Introduction

The epidermis is a dynamic structure with multiple functions, including barrier protection, immune surveillance, and thermoregulation. Under physiologic conditions, the epidermis maintains, through the ordered process of differentiation, four microanatomic compartments: basalis, spinosum, granulosum, corneum. The transition from a basilar keratinocyte to a corneocyte is a tightly regulated process balancing cell proliferation with cell loss and associated with a state of equilibrium termed epidermal homeostasis. Often, dermatologic diseases are correlated with perturbations in epidermal homeostasis, that are manifested histologically by changes in the stratum basalis, spinosum, granulosum, and corneum. Further characterization of epidermal homeostasis at the molecular level should provide insights into the pathogenesis of cutaneous diseases.

Psoriasis is a common, hyperproliferative cutaneous disorder associated with an inflammatory infiltrate containing T-lymphocytes and macrophages. The hyperproliferative epidermal changes in psoriasis are most likely secondary to localized increased production of cytokines, chemokines and growth factors.¹

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Squamous cell carcinoma and related precursor lesions represent common hyperproliferative disorders associated with aberrant keratinocyte differentiation. Squamous cell carcinoma and precursor lesions are associated with alterations in growth factor receptor signaling. However, direct characterization of growth factor-dependent signaling in human cutaneous SCC and related precursor lesions is incomplete.

Stimulation of many growth-factor receptor tyrosine kinases and chemokine receptors leads to the activation of Src-family tyrosine kinases (SFKs).² The SFKs consist of 8 homologous non-receptor tyrosine kinases including: Src, Fyn, Yes, Lck, Hck, Fgr, Lyn, and Blk.³ Src, Fyn, and Yes are widely expressed in many cell types, including keratinocytes. Activation of SFKs is tightly regulated and necessary for cell growth and differentiation, especially in keratinocytes.⁴ However, persistent activation of these kinases can result in cellular transformation.⁵ Activation of SFKs requires phosphorylation of a catalytic site tyrosine, tyrosine 416 in human Src. Recently, phospho-specific antibodies have been developed that specifically recognize this phosphorylated tyrosine; therefore, application of this pY416 antibody to tissue sections can be used to qualitatively assess the level of SFK activation in a biopsy sample. Given the biological importance of SFK activation in keratinocyte growth, the levels of activated SFKs in biopsies of psoriasis, SCC, SCIS, and actinic keratoses were evaluated. In this report, immunohistochemical analysis of biopsies of psoriasis, actinic keratoses, squamous cell carcinomas *in situ*, and invasive squamous cell carcinomas demonstrated elevated levels of activated SFKs compared to unremarkable epidermis. These data directly implicate activation of SFKs in the pathogenesis of psoriasis and cutaneous SCC.

Materials and Methods

Immunohistochemical Detection of Phosphorylated SFKs

Formalin-fixed, paraffin-embedded tissue samples of psoriasis, actinic keratoses, squamous cell carcinoma, and unremarkable epidermis were retrieved from the dermatopathology archives of Department of Dermatology, Univ. of Penn. Each specimen was reviewed and evaluated by RE and JTS then categorized as psoriasis (N=14), actinic keratosis (N=4), squamous cell carcinoma *in situ* (N=3), squamous cell carcinoma (N=4), and unremarkable epidermis (N=6). The samples were heated, de-paraffined, re-hydrated, and washed using standard procedures. Antigen retrieval was performed by incubating the slides in 10 mM citrate buffer (pH 6) at 85.0°C for 20 minutes; the container was removed and allowed to cool to room temperature over 30 minutes. The tissue sections were incubated at room temperature for 30 minutes with 10% goat serum to inhibit non-specific binding; sections were then incubated for 105 min at room temperature with a 1:25 dilution of rabbit anti-phospho-Src family (Tyr416) antibody (Cell Signaling Technology, Danvers, Ma, USA). Affinity-purified biotinylated goat anti-rabbit polyclonal immunoglobulin (BD Biosciences Pharmingen, USA) was applied to the tissue sections at a 1:50 dilution for 30 minutes. Following the secondary antibody incubation, pre-diluted Streptavidin-Horseradish Peroxidase (BD Biosciences Pharmingen, USA) was applied to the tissue sections and incubated for 30 minutes. Histochemical development was performed using a liquid 3, 3'-Diaminobenzidine tetrahydrochloride (DAB) substrate kit (Zymed Laboratories Inc., San Francisco, Ca, USA), for 12 minutes. The slides were subsequently counterstained in hematoxylin, dipped thrice in bluing reagent, dehydrated, and cleared. Comparisons of phosphorylated SFK levels were made by examining differences in degree of staining between the unremarkable and psoriasis, SCC, SCIS, or actinic keratosis samples. Slides were stained at least twice in a batch-wise manner to confirm comparative staining.

Results

All psoriasis specimens displayed significantly greater staining for activated SFKs when compared to sections of unremarkable skin (Fig. 1). Specific staining for activated SFKs was observed predominantly in a membrane pattern with a lesser degree of cytoplasmic staining; significant nuclear staining was not noted. In addition, one psoriasis sample contained portions of epidermis with a lesser degree of epidermal hyperplasia; these areas displayed significantly less staining for activated SFKs than areas of prominent psoriasiform hyperplasia (Fig. 1).

The degree of SFK activity was evaluated in lesions comprising the spectrum of cutaneous neoplasia: actinic keratoses (AKs), squamous cell carcinomas *in situ* (SCISs), and squamous cell carcinomas (SCCs). All AKs displayed significantly greater staining for activated SFKs than unremarkable skin (Fig. 2). Moreover, the AKs displayed less extensive and weaker staining than SCIS or SCC (Fig. 2). The SCIS lesions displayed uniform prominent and extensive staining for activated SFKs. No discernable difference was seen between the *in situ* and invasive specimens. Both membrane and cytosolic staining were noted in neoplastic lesions. Interestingly, no significant nuclear staining was seen.

Discussion

This report demonstrates that SFKs are activated in human biopsies of psoriasis, AKs, SCIS, and SCC. These cutaneous disorders exhibit altered keratinocyte differentiation and hyperproliferation and are associated with increased EGF receptor activity. Stimulation of the EGF receptor (EGFR) likely plays a role in activating SFKs in these hyperproliferative disorders because SFKs are directly activated by EGF in human keratinocytes. Although psoriasis and SCC both exhibit SFK activation, there must be additional molecular differences that account for the significant differences in clinical and biological behavior of these disorders.

The pathophysiologic model of psoriasis has recently undergone a paradigm shift from a T-cell mediated disease to one in which the keratinocytes play important roles in promoting the aberrant immune-reaction between keratinocytes and T-cells.^{6, 7} The hyperproliferative epidermis of psoriasis is likely secondary to increased expression of chemokines that activate intracellular signaling pathways promoting growth. The relationship between increased SFK activity in keratinocytes and cytokine production will be interesting to explore.

Cutaneous neoplasia is a multifactorial disease that results from activation of oncogenes and/or loss of tumor suppressor genes, ultimately leading to excessive cell growth. Our data supports the hypothesis that SFKs are key orchestrators of epidermal homeostasis, as activation of these kinases is seen in both inflammatory and neoplastic hyperproliferative disorders.

The increased SFK activity in psoriasis and cutaneous neoplasia provides evidence that pharmacologic targeting of SFKs may have therapeutic benefit for psoriasis and SCC. Inhibition of SFKs in psoriasis and SCC could plausibly resolve these diseases by downregulating the cellular activity of downstream growth-regulatory molecules. Interestingly, a recently discovered adaptor molecule called Srcasm, Src activating and signaling molecule, has been shown to regulate the activity of SFKs⁸. Additional work has shown that Srcasm is a promoter of keratinocyte differentiation and is decreased in cutaneous neoplasia^{9, 10}. Although the precise mechanism of Srcasm-dependent SFK downregulation is currently being studied, insights from these studies may provide insights into psoriasis and SCC pathogenesis.

Our study is the first to show activation of SFKs in human biopsy specimens of psoriasis and cutaneous neoplasia. This data supports the hypothesis that SFKs are important mediators of epidermal homeostasis. Assessing the levels of SFK activation in other epithelial hyperproliferative disorders may provide additional insights into these diseases.

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Figure 1. Immunohistochemical (IHC) staining for activated Src-family kinases in unremarkable skin and psoriasis

A, low level of staining in unremarkable epidermis. *B*, there is significantly greater membrane and cytoplasmic staining in the psoriasiform epidermis. *C*, area of mild hyperplasia exhibits lesser degree of staining compared to adjacent area of prominent hyperplasia, *D*.

Figure 2. Immunohistochemical detection of activated SFKs in AKs, SCISs, and SCCs
 Sections of actinic keratoses (AK), squamous cell carcinoma in situ (SCIS), and squamous cell carcinoma (SCC) were incubated with pY416 antibody to detect activated SFKs. There is a significantly greater degree of membrane and cytoplasmic staining in the AK, SCIS, and SCC compared to the unremarkable epidermis. Two magnifications of representative biopsies are shown.