
# Identification of tissue damage, extracellular matrix remodeling and bacterial challenge as common mechanisms associated with high-risk cutaneous squamous cell carcinomas

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## Abstract

In this study we used a genetic extracellular matrix (ECM) disease to identify mechanisms associated with aggressive behavior of cutaneous squamous cell carcinoma (cSCC). cSCC is one of the most common malignancies and usually has a good prognosis. However, some cSCCs recur or metastasize and cause significant morbidity and mortality. Known factors that are associated with aggressiveness of cSCCs include tumor grading, size, localization and microinvasive behavior. To investigate molecular mechanisms that influence biologic behavior we used global proteomic and histologic analyses of formalin-fixed paraffin-embedded tissue of primary human cSCCs. We compared three groups: non-recurring, non-metastasizing low-risk sporadic cSCCs; metastasizing sporadic cSCCs; and cSCCs from patients with recessive dystrophic epidermolysis bullosa (RDEB). RDEB is a genetic skin blistering and ECM disease caused by collagen VII deficiency. Patients commonly suffer from high-risk early onset cSCCs that frequently metastasize. The results indicate that different processes are associated with formation of RDEB cSCCs compared to sporadic cSCCs. Sporadic cSCCs show signs of UV damage, whereas RDEB cSCCs have higher mutational rates and display tissue damage, inflammation and subsequent remodeling of the dermal ECM as tumor initiating factors. Interestingly the two high-risk groups – high-risk metastasizing sporadic cSCCs and RDEB cSCCs – are both associated with tissue damage and ECM remodeling in gene-ontology enrichment and Search Tool for the Retrieval of Interacting Genes/Proteins analyses. In situ histologic analyses validate these results. The high-risk cSCCs also show signatures of enhanced bacterial challenge. Histologic analyses confirm correlation of bacterial colonization with worse prognosis. Collectively, this unbiased study – performed directly on human patient material – reveals that common microenvironmental alterations linked to ECM remodeling and increased bacterial challenges are denominators of high-risk cSCCs. The proteins identified here could serve as potential diagnostic markers and therapeutic targets in high-risk cSCCs.

## Introduction

Cutaneous squamous cell carcinoma (cSCC) is a common and potentially aggressive cancer. As the body's outermost barrier, the skin is continuously exposed to external chemical, physical, biological, and mechanical challenges. As a result, DNA damage progressively accumulates, resulting in a high mutational burden in aged skin. For example, mutations in the context-dependent *NOTCH1* and *2* onco/tumor suppressor genes are common in clinically unaffected aged skin [1,2]. Importantly, most mutations remain functionally silent, even when occurring in known oncogenes, and do not trigger malignant clonal expansion of affected keratinocytes [1]. This emphasizes that multiple mutation-independent events control tumorigenesis in the skin and puts a spotlight on the microenvironment as an essential regulator [3]. Non-melanoma skin cancer (NMSC) – arising from mutated keratinocytes – is the most common malignancy worldwide [4]. One fifth of NMSCs are cSCCs [4]. They usually have a good prognosis. However, some cSCCs recur or metastasize and cause significant morbidity and mortality [5]. The five-year recurrence and metastasis rate is under 10%. Yet 10-year survival of cSCCs metastasizing to regional lymph nodes is under 20% and under 10% in patients with distant metastasis [6]. The majority of cSCCs is UV-induced, and occurs on sun-exposed skin of elderly patients. cSCCs are more common in fairer skin phototypes. However, a subset of more aggressive cSCCs arises on sites with chronic tissue damage and inflammation [7]. In addition to causing DNA damage, prolonged exposure to UV light leads to dermal alterations, e.g. solar elastosis. Some of those changes also occur with aging alone. A natural function of the dermis is to limit epidermal activities. Thus, the increased occurrence of cSCCs in elderly patients could be explained by both higher frequency of driver mutations in epidermal stem cells, and UV- and age-induced cancer enabling remodeling of the dermis [3].

Cancer cell-intrinsic as well as microenvironmental factors determine the biologic behavior of cSCCs, but molecular mechanisms promoting systemically invasive and metastatic behavior are insufficiently understood [3]. Mutations in *NOTCH* and *TP53* are frequent in cSCCs [8]. Canonical TGFβ signaling restricts keratinocyte proliferation under physiological conditions. Mutations in *TGFBR1* and *TGFBR2* genes, leading to inactivation of TGFβ receptors 1 and 2, have been described as a promoter of cSCC development [8]. In this context, a facilitating microenvironment may allow for the unrestricted clonal expansion of transformed epidermal stem cells. However, these events occur in cSCCs of different biological behavior and thus are not directly correlated with progression to metastatic cancers. Clinically, known factors that correlate with aggressiveness of cSCCs include grading, size, localization and microinvasion. Tumor size positively correlates with metastasis risk [5]. Therefore it can be concluded that metastasis is a relatively late event in cSCC progression. It also indicates that primary tumors may be heterogeneous with only limited cell populations having metastatic capabilities. These observations suggest that bulk studies of primary tumors can help to understand the processes that promote aggressive biologic behavior of cSCCs.

Carcinomas arising in patients suffering from the severe skin blistering disorder recessive dystrophic epidermolysis bullosa (RDEB) are an illustrative example of microenvironment-propagated progression of cSCCs [9]. The disorder is caused by mutations in the *COL7A1* gene encoding the extracellular matrix (ECM) protein collagen VII, which anchors the epidermal basement membrane to the papillary dermal ECM [10]. Loss of collagen VII or reduced protein functionality leads to fragile skin and wounds that heal with excessive scarring [11]. As a consequence, severely affected individuals develop progressive soft tissue fibrosis at chronically injured sites [11,12]. The occurrence of metastatic and invasive cSCCs on such sites is a leading cause of mortality in patients with severe RDEB [9]. On histology, RDEB cSCCs are extremely well differentiated. Thus their frequently aggressive behavior is independent of dedifferentiation [13]. Furthermore, RDEB cSCCs largely display the same mutation spectrum as much less aggressive sporadic UV-induced cSCCs [8,14]. These data indicate that biological aggressiveness of RDEB cSCCs is largely influenced by the microenvironment. Aggressiveness is less dependent on epithelial-mesenchymal transition (EMT) and is independent of mutations. Indeed, two-stage chemical carcinogenesis with 7,12-dimethylbenz[*a*]anthracene, which induces homogenous *hRas* mutations, followed by tumor-promotion with 12-*O*-tetradecanoylphorbol-13-acetate, in RDEB mice results in cSCCs in contrast to papillomas in wild-type mice [15].

Uncovering mutation-independent processes that are associated with aggressive behavior of cSCCs could reveal valuable diagnostic markers and therapeutic targets. This would potentially allow for better treatment of a large number of patients. We reasoned that performing unbiased head-to-head comparison in different cSCC types would help to identify common processes regulating tumor progression. We compared three groups: non-recurring, non-metastasizing low-risk sporadic cSCCs; metastasizing sporadic cSCCs; and high-risk RDEB cSCCs, which commonly show aggressive potential.

Toward this end, we employed proteomic analysis of tissue sections from formalin-fixed paraffin-embedded (FFPE) human cSCCs. As expected, these analyses showed that processes associated with induction of cSCCs were different in RDEB cSCCs compared to sporadic cSCCs. RDEB cSCCs displayed signs of tissue damage and inflammation, sporadic cSCCs