Available online at www.sciencedirect.com
SCIENCE DIRECT
Pathology – Research and Practice 202 (2006) 599–607
PATHOLOGY RESEARCH AND PRACTICE
www.elsevier.de/prp

ORIGINAL ARTICLE

Expression of cell cycle and apoptosis regulatory proteins in keratoacanthoma and squamous cell carcinoma

Tanja Batinac[a], Gordana Zamolo[b,*], Miran Coklo[b], Ita Hadzisejdic[b], Christophe Stemberger[b], Gordana Zauhar[c]

[a]*Department of Dermatovenerology, Rijeka University Hospital, Rijeka, Croatia*
[b]*Department of Pathology, Rijeka University School of Medicine, Brace Branchetta 20, Rijeka 51000, Croatia*
[c]*Department of Physics, Rijeka University School of Medicine, Rijeka, Croatia*

Received 23 December 2005; accepted 11 April 2006

Abstract

Some authors view keratoacanthoma (KA) as a variant of squamous cell carcinoma (SCC), while others consider it a separate entity that must be distinguished from SCC. Involution displayed by KA is an important difference between these two entities. It has been suggested that apoptosis plays a role in the involution process of KA, although the exact trigger for it remains unclear.

A hundred and fifty specimens were included in this study, 30 cases for each of the following groups: normal skin (NS), proliferative keratoacanthoma (pKA), regressing keratoacanthoma (rKA), well-differentiated squamous cell carcinoma (wdSCC), and poorly differentiated squamous cell carcinoma (pdSCC). They were immunohistochemically examined for the expression of p53, Ki-67, bak, and bcl-2.

Significantly higher p53 and Ki-67 expressions were observed in all tumor lesions examined as compared with NS. There was higher bak expression in KAs compared to NS and a significant reduction of bak expression in pdSCC together with a significant reduction of bak expression in SCCs compared to pKA. Bcl-2 expression was similar in NS and SCCs, but was lower in rKA. We found a significant positive correlation between p53 and Ki-67, p53 and Bak in NS and examined skin tumors.

Lower bcl-2 expression in conjunction with higher bak expression in rKA suggests a possible role of these apoptosis-regulating proteins in tumor regression. In contrast to this finding, a steady level of bcl-2 expression in pdSCC combined with lower bak expression levels and a high proliferation rate could contribute to progression and aggressiveness in these tumors. Bak and p53 expression is a sun-related and age-dependent process in NS and skin tumors.

Keywords: Apoptosis; Bcl-2; Bak; Keratoacanthoma; Squamous cell carcinoma

Introduction

In the literature, there is much controversy about the question of whether keratoacanthoma (KA) is a variant of squamous cell carcinoma (SCC) or a unique lesion [20,36,48]. Although most of the lesions that clinically

*Corresponding author. Tel.: +385 51 325 813; fax: +385 51 325 810.
E-mail address: gordanazamolo@yahoo.com (G. Zamolo).

0344-0338/$ - see front matter © 2006 Elsevier GmbH. All rights reserved.
doi:10.1016/j.prp.2006.04.004

and/or histologically fit the diagnosis of KA behave in a predictably benign manner, occasional aggressive behavior, including metastases, has been described [12,28,48]. Clinically, KAs arise rapidly, typically on sun-exposed skin, to form a dome-shaped papule within 4–6 weeks on average. The lesions usually regress over the next 4–6 months, leaving a depressed scar [20,36,48]. Involution displayed by KA is a single feature that separates KA from wdSCC.

It has become clear that molecular events regulating cell survival, apoptosis, growth arrest, and cell differentiation are important contributors to the overall kinetics of benign and malignant cell growth and play a role in the development, progression, and regression of benign and malignant cell growth [2,19,45]. Also, immunological mechanisms have been implicated in a phenomenon of spontaneous tumor regression [5,31].

Protein p53 is a well-described tumor suppressor that has a central role in the initiation of apoptosis and in cell cycle control [13,25]. Its critical role in maintaining integrity of the human genome is evident, because p53 is the most commonly altered gene in human cancer [2,25,45]. Due to a short half-life *in vivo*, p53 is usually undetectable in normal skin (NS). Since an association between p53 protein expression and cell proliferation has been suggested [2], Ki-67 staining was performed to evaluate the cell cycle status. Ki-67 is a nuclear antigen expressed in actively cycling cells but not in resting G0 cells, and is frequently used as a marker for cell proliferation in tissue sections [4].

Several proteins of the bcl-2 gene family have been implicated in the regulation of programmed cell death and cell proliferation [7,24,43,45]. The pro-survival proteins, such as bcl-2/bcl-x, block apoptosis, whereas Bax-like proteins bax and bak induce apoptosis [7,24]. The list of molecular mediators influencing apoptosis is expanding rapidly. Bcl-2 and its homologous proteins have emerged as the most important regulators of programmed cell death, playing a crucial role in the balance between cell survival and cell death. Some authors believe that bak protein induces apoptosis in various cell types by opposing the protecting function of bcl-2 and bcl-xL [14,49].

Recent studies have suggested that the proapoptotic proteins bax and bak are the effectors that execute cell death by antagonizing the ability of Bcl-2 family members, such as bcl-2 and bcl-xL, to suppress apoptosis, whereas bcl-2 and bcl-xL bind to bak and bax to inhibit bak/bax-mediated apoptosis [7,14,24,49]. The balance between these two groups can be disrupted by the BH3-only proteins, proposed alosteric regulators of the Bcl-2 family of proteins that serve as central effectors of apoptotic signaling pathways [7,49].

To gain an insight into the molecular pathogenesis and the role of cell cycle and apoptosis regulatory proteins in the regression of KA and progression of SCC, we examined the expression and distribution of Bcl-2 family proteins, pro-apoptotic (bak) and anti-apoptotic (bcl-2), as well as the expression of p53 and Ki-67 proteins.

Materials and methods

Patients and skin specimens

A hundred and fifty skin specimens were obtained from the Department of Dermatovenerology, University Hospital Rijeka, and the Department of Pathology, Faculty of Medicine, University of Rijeka, Croatia, between 2000 and 2005. Specimens included NS, proliferative keratoacanthoma (pKA), regressing keratoacanthoma (rKA), well-differentiated (wdSCC) and poorly differentiated squamous cell carcinoma (pdSCC), 30 cases assigned to each group. In this study, we classified SCCs into two categories according to Broder's grading system; well (Broder's grade I) and poorly (Broder's grade III) differentiated type. NS samples were obtained from NS surrounding fibromas or hemangiomas surgically resected for cosmetic reasons.

Tissues were obtained from 82 (54.67%) men and 68 (45.33%) women. The average age of the patients was 69.10 years (range 48–89 years), 67.87 years (range 49–86 years), 75.50 years (range 60–91 years), 76.57 years (range 60–92 years), and 65.86 years (range 39–87 years) for pKA, rKA, wdSCC, pdSCC, and healthy controls, respectively. All specimens were fixed in 10% buffered formaldehyde and embedded in paraffin. Sections (4 μm) were stained with hematoxylin-eosin, and two pathologists examined each slide independently.

Histology

Both pKA and rKA were histologically determined by the presence of an exoendophytoc squamous proliferation with a central, keratin-filled crater, the overlying epidermis extending around the crater, forming "lips". Keratinocytes underlying keratin-filled crater had eosinophilic or glassy cytoplasm. The pKA was composed predominantly of the well-differentiated proliferative epithelium enveloping a small, keratin-filled crater surrounded by moderate to intense lymphohistiocytic inflammation at the base. Cells displayed moderate pleomorphism with occasional to numerous mitoses. With progressive regression of the lesion (rKA), the keratin-filled crater was diminished, and the proliferating epithelium tended to flatten out, leaving a somewhat papillomatous base with underlying less intense, chronic inflammation and fibrosis. Neoplastic keratinocytes displayed minimal pleomorphism and rare mitoses.