Immunohistochemical determination of the growth hormone receptor in squamous cell carcinoma of the skin

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ABSTRACT

Background. The growth hormone (GH) that regulates the metabolism, cell differentiation and growth, functions by acting on specific growth hormone receptors (GHR). As GHR have been detected in different types of human neoplasms, we wanted to determine GHR expression in squamous cell carcinoma of the skin (SCC).

Materials and methods. An immunohistochemical technique was used to demonstrate the presence of GHR in 27 SCC specimens (grade G1) and the surrounding perilesional normal skin (PNS). The relative proportion of immunoreactive cells was counted following the semiquantitative method.

Results. There was an uniform GHR expression in PNS that presented itself as a weak positivity in all specimens. Among 27 SCC cases studied, 93% were immunoreactive: 18 were strongly positive, 5 moderately positive, 2 weakly positive and 2 negative.

Discussion. Our results confirm previous observations that neoplastic cells in skin tumors are directly responsive to GH action. For this reason GH could be involved through GHR in the development of cutaneous neoplasms.

KEY WORDS

growth hormone receptor, immunohistochemistry, skin squamous cell carcinoma

Introduction

Skin squamous cell carcinoma (SCC) is, after basal cell carcinoma, the second most common non-melanoma skin cancer (1). Since the 1960s, the incidence of SCC has been increasing at a rate of 4% - 8% per year (2). Escalating SCC incidence is attributed to increased exposure of the population to solar UV radiation (1,3,4).

The most important cause of SCC is long-term UV exposure, i.e. photocarcinogenesis (5). Various mutations of SCC caused by UV light are thought to induce expansion of keratinocyte clones, which acquire additional mutations and progress into SCC (6,7). Although many investigations have been made, the detailed molecular mechanism of SCC development and progression is still unclear.

The growth hormone (GH) is the major hormone regulating somatic and skeletal growth and bone maturation. GH also plays a crucial role in controlling the metabolism and acts throughout the body to promote

not only balanced growth, but also differentiated cell expression. The skin has been recognized as one of the target tissues for GH after findings of alterations in skin texture and thickness in clinical states of both GH deficiency and excess. In acromegaly, the skin is thickened through increment of the seborrheic secretion, the amount of dermal tissue and the number of skin tags (8). In the reverse case, dwarfism, the skin is thin and has undergone a loss of elasticity (8).

Actions of GH on target tissue are mediated through binding to the GH receptors (GHR). In skin, GHR are detectable on the mRNA and protein level, which implies that GH has a direct effect on skin growth and function *in vivo* (9-15).

Investigation done by Lincoln et al. (12) reported GHR expression in a great variety of neoplastic cells and thus implicated both GH and GHR in the development and/or progression of those tumors. For the purposes of this study, Melanoma was the only skin tumor investigated. GHR was strongly expressed in 48 out of 76 naevi (63%), and in 49 of 50 (98%) of the malignant melanomas investigated (12). Ginarte et al. (13) have investigated GHR expression in a number of the most frequent proliferative benign and malignant cutaneous entities. Seven SCC specimens were included in this investigation. Although GHR immunoreactivity was detected in all SCC specimens, it has always been found weaker than in normal keratinocytes (13).

Finally, two investigations from our laboratory investigated GHR positivity in actinic keratosis (AK) (14,15). AK is a precancerous cutaneous lesion, which was recently recognized as an early stage in the biological continuum that leads from carcinoma *in situ* to invasive squamous cell carcinoma (SCC) (1,5,7). We had detected GHR immunopositivity in 12 out of 33 (36%) specimens of hypertrophic AK type (14) and in 20 out of 25 (80%) specimens of atrophic AK type (15).

This investigation evaluates GHR expression in well-differentiated SCC (grade G1) and the surrounding perilesional normal skin (PNS). Also, GHR expression is correlated to clinical parameters: age, sex and lesion size.

Patients and methods

Tissue samples

We investigated GHR expression in 27 specimens of well-differentiated SCC (grade G1) and in the surrounding perilesional normal skin (PNS). All 27 specimens, obtained from the files of the Ljudevit Jurak University Department of Pathology, Sisters of Charity University Hospital, Zagreb, Croatia, were from regularly sun-exposed skin in patients of both sexes, and older than 60 years of age. Clinical information about the patients (age, sex, lesion size and location) was obtained from departmental charts. The histopathologic diagnosis was established on routine sections stained with hematoxylin and eosin, and using well established criteria: the irregular mass of epidermal cells that proliferate into the dermis; the atypicality of tumor cells (the great variety of shape and size, hyperplasia and hyperchromasia of the nuclei; the absence of intercellular bridges and the presence of atypical mitotic figures) and the presence of keratinisation within the carcinoma, usually resulting in horn pearls (Figure 1.). Each specimen was re-evaluated by three expert pathologists (H.Č., B.K. and M.B.).

Table I. Growth hormone receptor (GHR) *expression in squamous cell carcinoma of the skin (SCC) and surrounding perilesional normal skin (PNS) (n=27).*

GHR expression	PNS		GHR	
-	0	0%	2	7%
+	27	100%	2	7%
++	0	0%	5	19%
+++	0	0%	18	67%
Total positive	**27**	**100%**	**25**	**93%**

(-) no positive cells
(+) less than 10% positive cells
(++) 10 to 25% positive cells
(+++) more than 25% positive cells

Immunohistochemical detection of GHR proteins

For immunohistochemical (IHC) determination of GHR expression serial 5 μm sections were cut from the paraffin blocks and collected on polylysine-coated slides. The presence of GHR was demonstrated, after deparaffinisation, by the streptavidin-biotin horseradish peroxidase complex (Strept ABC-HRP) technique that has been described elsewhere (13,14). Liver tissue was used as a positive control.

Data evaluation

The positive cells were scored in several randomly selected fields at x400 magnification. Only clear cytoplasmic GHR positivity was scored. Expression of immunohistochemical staining was represented as a percentage of immunoreactive cells aross 1000 analysed cells. The following semi-quantitative evaluation and scoring system was used: (-) for no immunoreactive tumour cells; weakly positive (+) for up to 10% of immunoreactive tumour cells; moderately positive (++) for 10% to 25% of immunoreactive tumour cells; and strongly positive (+++) for more than 25% of immunoreactive tumour cells. Cytotopographic localization (cytoplasmatic or nuclear) of immunohistochemical staining was also analysed.

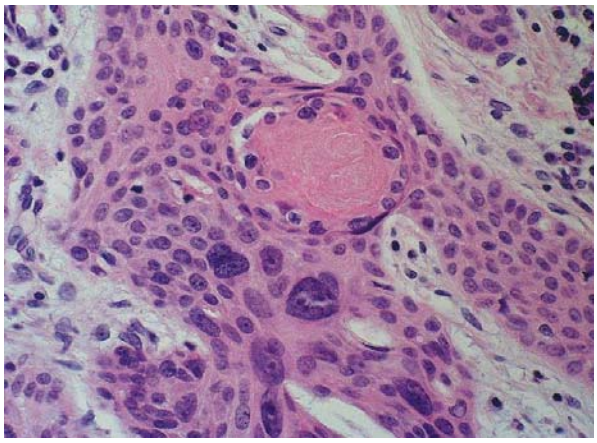

Figure 1. Hematoxylin and eosin staining of skin squamous cell carcinoma, grade I (x400).

The statistical analysis was performed by the statistical program GraphPad InStat version 3.00, GraphPad Software, San Diego, SAD using the χ^2-test for trend, the Fisher-exact test and the ANOVA test. The level of significance was set at $p < 0.05$ in all cases.

Results

Growth hormone receptor expression in PNS and SCC

There was a uniform expression of GHR protein in PNS: weakly positive (+) in all cases examined. Immunopositive keratinocytes were localized mainly in the lower layers of the epidermis (especially the basal epidermal layer) (Figure 2). In contrast, the majority of investigated SCC specimens (67%) showed strong (+++) GHR positivity (Table 1). Immunoreactive keratinocytes were spread diffusely over the positive SCC lesions. Well-differentiated tumor cells in proximity to the keratin pearls showed more intense immunopositivity than undifferentiated tumor cells (Figure 3). GHR expression in the SCC specimens was significantly increased compared to the PNS specimens (χ^2-test, $p<0.05$).

Comparison of GHR expression in SCC in relation to clinical parameters

GHR expression did not show any relationship to other clinical parameters such as lesion size, age (ANOVA, $p>0.05$) and sex (χ^2 test, $p>0.05$).

Discussion

Our study revealed a GHR immunoreactivity of the skin layers and adnexal structures that showed a similar pattern as has been reported elsewhere (9-15). GHR positivity was present both in cytoplasm and nucleus, as reported elsewhere (12,13). This interesting finding can be explained in two ways: Either, that nuclear staining originates from the presence of residual GHR epitopes in the course of their degradation, or that another type of GH receptor exists in the nucleus, along with the GHR in the membrane (13).

The majority of investigated SCC specimens (93%) showed GHR immunoreactivity. A similar proportion of positive cases has also been reported elsewhere (13). The most intense reaction was obtained from well-differentiated parts of the tumour tissue, in proximity to keratin pearls. This finding is in accordance with previ-

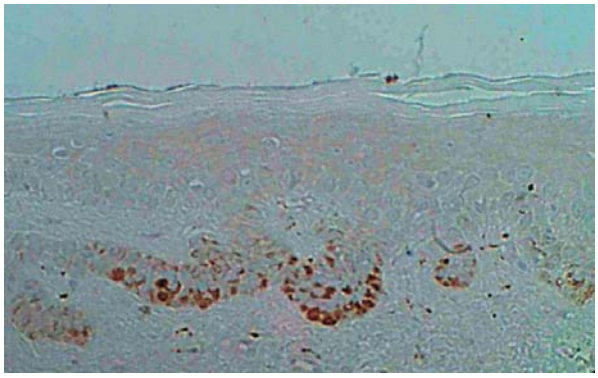

Figure 2. Immunohistochemical staining of perilesional normal skin for growth hormone receptor (Strept ABC-HRP, x200).

ous observations, which pointed out the correlation between GHR immunoreactivity and the degree of cellular differentiation: cells that are more differentiated showing more intense immunostaining than undifferentiated cells (13). However, differences in the intensity of GHR expression were detected. Our specimens of tumor tissue revealed stronger immunoreactivity than normal keratinocytes, while Ginarte and al. (13) ob-

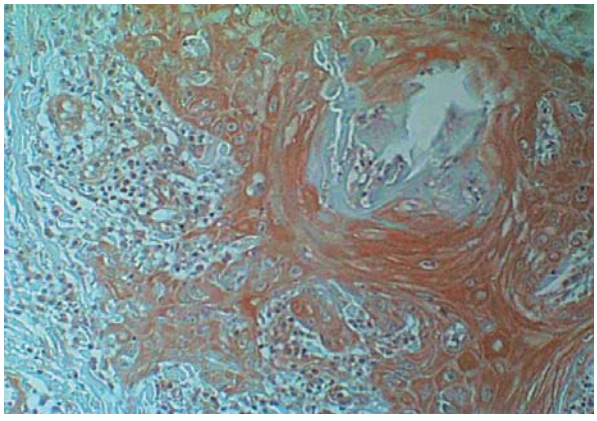

Figure 3. Immunohistochemical staining of skin squamous cell carcinoma, grade I, for growth hormone receptor (Strept ABC-HRP, x400).

served weaker immunopositivity of tumor cells than normal keratinocytes.

Our earlier investigation of hypertrophic acrtinic keratoses (HAK) determined GHR in 36% of the investigated HAK specimens and GHR positivity was significantly lower than in PNS (14). In AAK GHR positivity was detected in 80% of investigated lesions and statistical correlation showed no difference in the incidence of GHR positivity between atrophic actinic keratoses (AAK) and PNS (15). Furthermore, 32% of the investigated AAK specimens and 24% of the investigated HAK specimens showed increased GHR expression compared with PNS (14,15). As this investigation detected significantly increased GHR expression in SCC compared to PNS, we are tempted to conclude that those AK with an increased GHR expression may have an increased tendency to proliferate, and be in progression towards SCC.

In conclusion, these findings support the opinion that GHR is a ubiquitous receptor in normal skin and proliferative cutaneous lesions (12,13). A growth hormone (GH) may be involved in the development of different kinds of cutaneous precanceroses and neoplasms. In order to further clarify the role of GH/GHR in the development and progression of cutaneous precancerosis (AK) and neoplasm (SCC) it seems necessary to analyse larger groups of patients with different AK types or SCC grades.

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