Expression of heparanase in basal cell carcinoma and squamous cell carcinoma*

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Abstract: Background: Heparanase is an enzyme that cleaves heparan sulfate chains. Oligosaccharides generated by heparanase induce tumor progression. Basal cell carcinoma and squamous cell carcinoma comprise types of nonmelanoma skin cancer. Objectives: Evaluate the glycosaminoglycans profile and expression of heparanase in two human cell lines established in culture, immortalized skin keratinocyte (HaCaT) and squamous cell carcinoma (A431) and also investigate the expression of heparanase in basal cell carcinoma, squamous cell carcinoma and eyelid skin of individuals not affected by the disease (control). Methods: Glycosaminoglycans were quantified by electrophoresis and indirect ELISA method. The heparanase expression was analyzed by quantitative RT-PCR (qRTPCR). Results: The A431 strain showed significant increase in the sulfated glycosaminoglycans, increased heparanase expression and decreased hyaluronic acid, comparing to the HaCaT lineage. The mRNA expression of heparanase was significantly higher in Basal cell carcinoma and squamous cell carcinoma compared with control skin samples. It was also observed increased heparanase expression in squamous cell carcinoma compared to the Basal cell carcinoma. Conclusion: The glycosaminoglycans profile, as well as heparanase expression are different between HaCaT and A431 cell lines. The increased expression of heparanase in Basal cell carcinoma and squamous cell carcinoma suggests that this enzyme could be a marker for the diagnosis of such types of non-melanoma cancers, and may be useful as a target molecule for future alternative treatment.

Keywords: Carcinoma, basal cell; Carcinoma, squamous cell; Glycosaminoglycans; Hyaluronic acid; Neoplasms; Polymerase chain reaction; Skin neoplasms

INTRODUCTION

According to data from the National Cancer Institute (INCA), nonmelanoma skin cancer is the most common cancer in Brazil. It corresponds to 33% of all malignant tumors in the country, and presents low mortality and high cure rates when detected early on. Of the types of nonmelanoma skin cancer, basal cell carcinoma (BCC) is the most common, responsible for 70% of the diagnoses, while squamous cell carcinoma (SCC), is responsible for approximately 25% of the cases. These tumors present differences in behavior, growth, and metastatic capacity.[1] Both BCC and SCC present good prognoses, especially if detected in their initial stages.[2]

The BCC consisting of cells that resemble epidermal basal cells is the least aggressive of the types of skin cancer.[3] BCC is a tumor with a low degree of malignancy, with the capability of local invasion, tissue destruction, recurrence, and a limited potential of metastasis.[4] BCC is formed by the atypical proliferation of squamous cells, of an invader nature, which can provoke metastasis.[5] SCC presents a considerable potential for recurrence, which is associated with the size of the tumor, degree of histological differentiation, depth of the lesion, perineural invasion, state of the patient's immune system, and anatomic detection.[6]

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Individuals that develop BCC present a high risk of developing new foci of basal carcinomas, as well as other types of skin cancer, such as melanomas and SCCs.[7]

Exposure to ultraviolet radiation is the main risk factor associated with the genesis of BCC and SCC, which is evident due to its greater occurrence when exposed to sunlight.[8] Studies suggest that the exposure to chronic UVB radiation activates heparanase, leading to the degradation of the heparin sulfate in the basal membrane and the increase in the interaction between the growth factor of the epidermis and the dermis.[9]

The skin itself contains a large quantity of hyaluronic acid (HA), dermatan sulfate (DS), heparan sulfate (HS), and keratan sulfate (KS), which modulate adhesion, migration, and cell proliferation processes.[10-12]

The sulfated glycosaminoglycan include chondroitin sulfate (CS), DS, KS, heparin (HEP), and HS, while the hyaluronic acid represents a non-sulfate GAG class.[13] The GAG can interact with distinct proteins, including chemokines, cytokines, growth factors, enzymes, and adhesion molecules, promoting the regulation of diverse biological functions.[10-14]

Proteoglycans are macromolecules made up of a protein skeleton linked to GAG strains. Proteoglycans are present on the cell's surface, intracellular granules, and the extracellular matrix, which can regulate cytokines, angiogenic factors, and growth factors. The effects of proteoglycans in various cell mechanisms in general are modulated by interactions with GAG strains or by interactions with the protein skeleton. Proteoglycans play an important role in the organization of collagen fibers and participate in biological phenomena, such as differentiation, maintenance, and organization of the extracellular matrix.[15] HS proteoglycans are essential components of the extracellular matrix and basal membrane, responsible for the integrity of the membrane and the barrier function.[16,17] HPSE has the capacity to cleave proteoglycan strains, facilitating invasion and metastasis of the tumor cells, generating oligossacharides that increase the activity of angiogenic factors, cytokines, and growth factors, thus inducing cell proliferation, migration and inflammatory responses.[18,19] The composition of the extracellular matrix is associated with the infiltration of metastatic tumor cells and inflammatory cells. [20]

The present study sought to compare the profile of GAGs and the mRNA expression of the HPSE enzyme in SCC cell strains (A431) and non-neoplasic strains (HaCaT), as well as investigate the HPSE expression in BCC and SCC samples from surgical resections in order to compare the results between such groups and control tissues from skin obtained from plastic surgery, by means of Blepharoplasty, analyzing possible correlations between the HPSE expression and the occurrence of BCC and SCC.

METHODS

Patients

This study analyzed 30 patient skin tissue samples, with no restrictions as to race, age, or gender. To evaluate the mRN expression of HPSE, the quantitative RT-PCR (qRT-PCR) method was applied. The samples were obtained from the Surgery Ward of the Dermatology Department of the ABC Medical School, retrieved from dermatological surgeries that had been previously recommended by this institution's outpatient service. The samples were divided into three groups, 10 samples of SCC, 10 samples of BCC, and 10 samples of non-neoplasic skin tissue received from blepharoplasty plastic surgery, which were used as the control tissues (CTR). The collection of tissue samples were performed using a 2 mm punch and all samples in this study were stored in liquid nitrogen for processing. The procedures described in this study were approved by the Research Ethics Committee from the ABC Medical School, registered under protocol number 041/2011.

Cell strains

This study used cell strains from human keratinocytes (HaCaT) and human SCC cells (A431). The strains defined as HaCaT and A431 were cultivated in a DMEM sterile culture medium, containing 10% bovine fetal serum (FBS) and antibiotics (100 µg/mL of streptmicin and 100 Ul/mL of penicillin).

Enzymatic degradation

The defining of galactosaminoglycans (CS and DS) was obtained after enzymatic degradation with specific lyasis; chondriotinase AC, which specifically degrades chondroitin sulfate; and chondroitinase ABS, which degrades CS and DS. The identification of the GAGs that have been synthesized and secreted into the culture medium was conducted by means of electrophoresis in a 0.55% agarose gel in a 1,3-diaminopropane acetate (PDA), 0.05M, pH 9.0, 100 Volts, for one hour, in a cooler at 4ºC (Dietrich 1976). After electrophoresis had been performed, the GAGs were precipitated in agarose gel in a 0.1% cetyltrymethylammonium solution (Cetavlon) for 2 hours. The gel was dried under ventilation and heat, and was later stained with a 0.1% toluidine blue stain in 1% acetic acid and 50% ethanol. The excess stain was removed by rinsing with a bleaching solution (1% acetic acid, 50% ethanol). The stained gel, dried at room temperature, was submitted to radioautography by exposure to an X-ray. The sensibilized film was then submitted to scanning in a Cyclone™ device (Packard Instrument Company, Meriden, CT, USA), showing the S-GAGs^{35} by scintillations per minute (spm)

HA dose

The fluorimetric method was applied to determine the HA described by Martins *et al*.[21] The quantification of HA from each sample was determined in values expressed by the µg of HA / µg of total proteins.

mRNA extraction, cDNA synthesis, and HPSE expression

The skin samples were submitted to mRNA extraction using the RNAspin kit (GE Healthcare®), following manufacturer instructions. The reverse transcription was performed by applying the protocol described by the manufacturer as of 5µg of total RNA. The mixture was incubated at 70ºC, for 10 minutes. Next, 4 µL of 5X buffer solution, 2 µl of dithiothreitol 0.1 mM of Promega®, 1 µl of deoxynucleotide triphosphate, 10 mM of Promega®, and 1 µL of reverse transcriptase enzyme (Promega®) was added to the mixture. This solution was incubated for 10 minutes at 25ºC, 50 minutes at 42ºC, and 10 minutes at 70°C to obtain the cDNA.

qRT- PCR

The qRT-PCR method allows for the definition of the relative mRNA expression of HSPE, which was achieved by using the sense oligonucleotide primer 5′ TGGCAAGAAGGTCTGGTTAGGAGA 3′ and antisense oligonucleotide primer 5′ GCAAAGGTGTCGGATAGCAAGG 3′. The amplification was performed using the SYBR® Green PCR Master Mix Reagent (Applied Biosystems, Carlsbad, California, USA), according to the following modified protocol: 1.5 µL of forward primer at 1.5 µM, 1.5 µL of reverse primer at 1.5 µM, 3 µL of cDNA 1:10, and 6 µL of SYBR® Green Master Mix 2X. The mRNA expression of HSPE was presented in relation to the geometric average of the endogenous constitutive gene expression (-ΔCt): ribosomal protein 18S L13A (RPL13a), sense oligonucleotide primer 5´TTGAGGACCTCT GTGTATTTGTCAA3´, antisense oligonucleotide primer 5´CCTGGAGGAGAAGAGGAAAGAGA3´, and the enzyme glyceraldehyde-3-phosphate dehydrogenase (GAPDH), sense oligonucleotide primer 5´TCGACAGTCAGCCGCATCTTCTTT3´ and antisense oligonucleotide primer 5´GCCCAATACGACCAAAT CCGTTGA3´. All of the trials were conducted in triplicate. The ABI PRISM 7000 SDS technological platform was used under the following thermocycling conditions: 95ºC for 10 minutes, 45 cycles at 95ºC for 30 seconds and at 60ºC for 1 minute, resulting in an approximate reaction time of 2 hours.

Statistical Analysis

The statistical analysis was performed using the Prism5 program for Windows (GraphPad Prism® Software Inc., CA, USA). All variables were considered to be non-parametric, in accordance with the Kolmogorov-Smirnov test. When comparing the two groups, the Mann-Whitney test was applied, and to compare more than two groups, the Kruskal Wallis test was applied, followed by the Dunn post-hoc test. For the analyses, the quantitative variables were described by average and standard deviation, while the significance level was set as p <0.05.

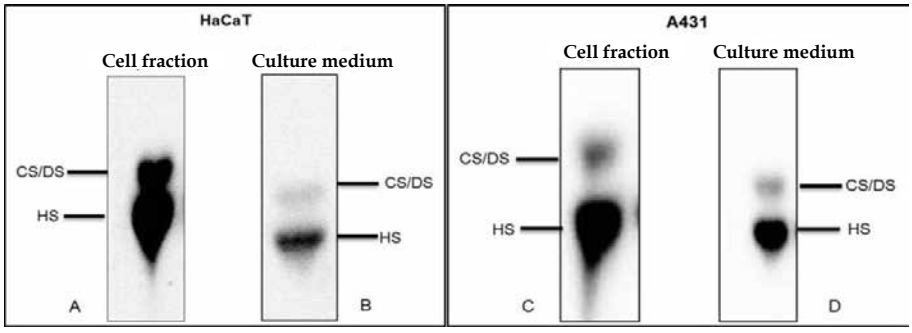
RESULTS

This study began by investigating the presence of sulfated GAG, by electrophoresis, in HaCaT and A431 cell strains. According to that illustrated in graph 1, we observed that the HaCaT and A431 cells presented a compound of electrophoretic migration that resembled CS/DS and another band corresponding to HS.

The definition of the type of CS and/or DS was determined after enzymatic degradation with specific lyasis, chondroitase AC, and chondroitase ABC, as presented in graph 2. The analysis of the enzymatic digestion with chrondroitase illustrated the presence of DS, which had been synthesized and secreted into a culture medium of both cell strains, HaCaT and A431 (Graph 2).

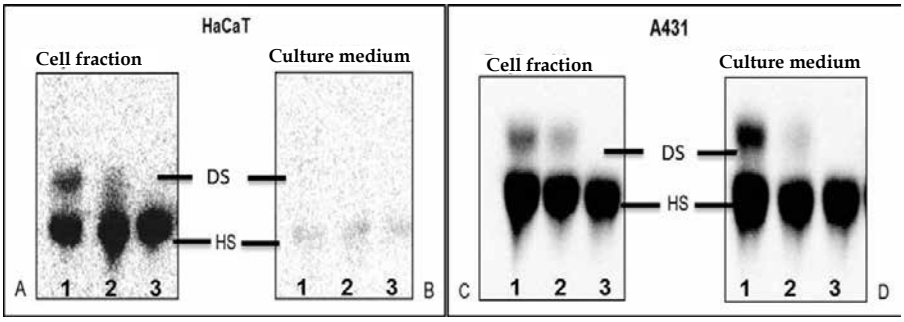
Graph 3 shows the results obtained from the quantification of DS and HS, synthesized by the HaCaT and A431 cell strains.

As observed in graph 3, the HS expression was significantly greater in the A431 cells, when compared to the HaCaT cells, respectively: 119030 ± 20775 cpm / µg total protein and 21731.25 ± 831.25 cpm / µg total protein, for the cell fraction (p = 0.0022, non-paired t test) and 94835 ± 18669 cpm / µg total protein 2787 ±50 cpm / µg total protein, for the HS secreted into the medium (p < 0.0001, non-paired t test). The DS values also presented significant



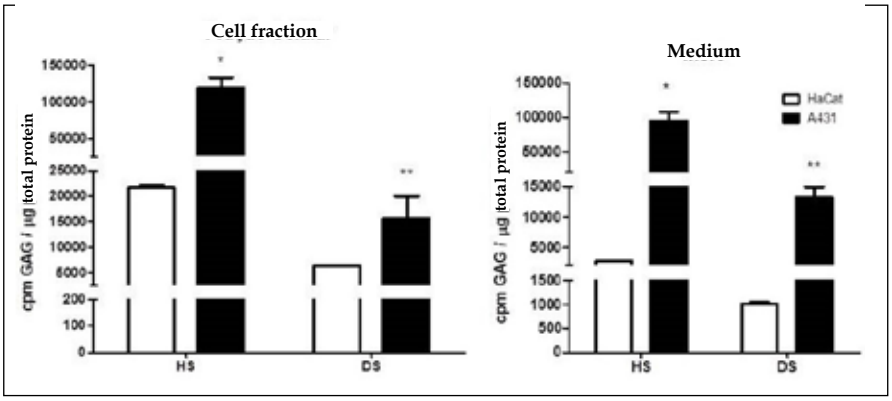
Graph 1:
Profile of glycosaminoglycan sulfate synthesized by HaCaT and A431

This trial shows the radioautography of electrophoresis conducted to identify and quantify the glycosaminoglycan (GAG) sulfates in the HaCat and A431 strains. HS, heparan sulfate; CS/DS, chondroitin sulfate and dermatan sulfate



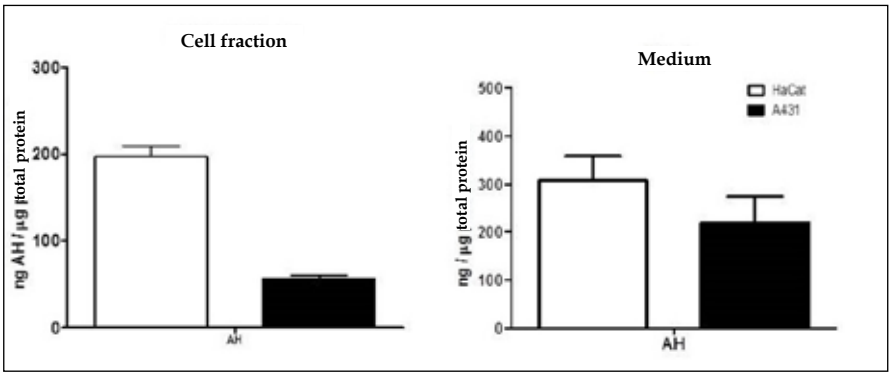
Graph 2:
Radioautography of electrophoresis after the enzymatic degradation of the GAG sulfates synthesized by HaCaT and A431 cells

This figure illustrates the presence of synthesized and secreted DS in both cell strains. The cells were marked with [35S]-sulfate. The extract obtained from the cell fraction (cells and extracellular matrix) and conditioned medium were submitted to degradation with chondroitinase AC and ABC, 1, samples not submitted to enzymatic degradation; 2, samples digested with chondroitinase AC, and 3, samples digested with chondroitinase ABC. HS, heparan sulfate and DS, dermatan sulfate



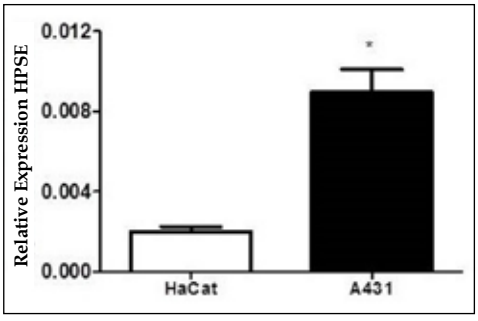
Graph 3:
Quantification of GAG sulfates in HaCaT and A431cells

HS, heparan sulfate; DS, Dermatan sulfate. Cell fractions, quantification of GAGs synthesized by cells and the extracellular matrix (*P = 0.0022 and **P = 0.0007, non-paired t test). Medium, GAGs secreted into the culture medium (*P < 0.0001 and **P = 0.0013, non-paired t test)



Graph 4:
Quantification of hyaluronic acid (HA) synthesized by HaCaT and A431 cells

HA, Hyaluronic acid. Cell fraction, quantification of HA synthesized by cells and the extracellular matrix; Medium, HA secreted into the culture medium. (*P = 0.0308, non-paired t test).



Graph 5:
Heparanase Expression in different cell strains

mRNA expression of HPSE obtained from qRT-PCR, as described in the Methods section. The values represent the relative expression of HPSE, using endogenous genes as the control (GAPDH, glyceraldehyde-3-phosphate-dehydrogenase, and RPL13a, ribosomal protein). HaCat, non-neoplasic human skin cell strain, and A431, human SCC neoplasic cell strain. The values represent the average and standard deviation of trials performed in triplicate. * P = 0.0048

differences when comparing both the A431 and HaCaT cells, respectively: 15602 ± 6134 versus 6362 ±137 in the cell fraction (p = 0.0007, non-paired t test) and 13219 ± 2418,87 versus 1011 ± 50.00 versus for the DS secreted into the medium (p = 0.0013, non-paired t test). Therefore, it is clear that the tumor cells (A431) synthesize and secrete larger quantities of HS and DS when compared to the HaCaT non-neoplasic cells, as shown in graph 3.

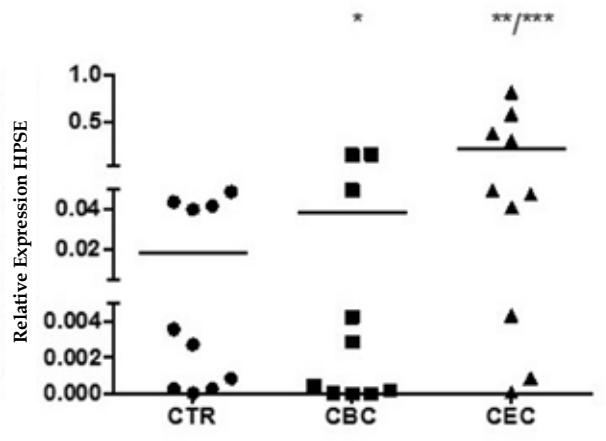
The quantification of the non-sulfate GAG, HA, was also defined in the HaCaT and A431 cell strains (Graph 4).

Graph 4 presents evidence of the significantly larger quantity of HA in the cell fraction of the HaCaT strain when compared to the A431 strain, respectively (196.1 ± 12.7 ng / µg total protein) versus (56.0 ± 4.3 ng / µg total protein) (p= 0.0308, non-paired t test). However, no difference in the quantity of HA secreted into the culture medium, when compared to HaCaT, was observed (306.2 ± 52.3 ng / µg total protein) versus A431 (218.4 ± 55 ng / µg total protein).

The mRNA expression of HSPE, from the HaCaT and A431 strains is presented in graph 5 and shows the increase in relative expression of HPSE in A431 tumor cells, 0.00897 ± 0.00103, as compared to the HaCaT non-neoplasic strain, 0.00199 ± 0.00028, (p = 0.0048).

Taking into account the results obtained in the analyses of both human skin cell strains (HaCaT and A431), we decided to investigate the expression of the HPSE enzyme in BCC and SCC samples, in comparison with skin from the eyelids of individuals who had not been diagnosed with any type of neoplasia (blepharoplasty).

Graph 6 illustrates that SCC and BCC, as compared to the blepharoplasty samples, presented an increased mRNA expression of the HPSE enzyme,. A significant difference was observed between the mRNA of the HPSE when compared to the control and SCC samples, respectively: (0.01827 ± 0.02204) and (0.2251 ± 0.2921), applying the Mann-Whitney test with a p<0.0001. In addition, the heparanase mRNA was also significantly higher in SCC (0.2251 ± 0.2921), as compared to BCC (0.03881 ± 0.06836), p = 0.0002. Nonetheless, a statistically significant difference between the control samples and patient tissues as regards SCC was identified (p=0.0024).



Graph 6: Relative mRNA expression of HPSE. The results were obtained by analysis using qRT-PCR, as described in the Methods section. CTR, samples collected from patients that presented no neoplasias (Control); BCC, sample of basal cell carcinoma; and SCC, samples from squamous cell carcinoma. The relative expression of HPSE was obtained using the endogenous genes GAPDH and RPL13a. The strains represent the average of the values of HPSE expression in each group. The values were collected through trials performed in triplicate. CTR versus BCC *P=0.0024; CTR versus SCC **P<0.0001, and BCC versus SCC ***P = 0.0002 (non-paired t test)

DISCUSSION

The GAG sulfates play an important role in cell signaling and in the remodeling of the extracellular matrix. According to the cell type, there is a broad structural variability of GAG sulfates. Changes in the profile of GAG sulfates are related to the development of many illnesses, such as cancer, inflammatory diseases, degenerative diseases, as well as healing processes.[22-25] The study of extracellular components, such as proteoglycans, fibrous proteins, glycoproteins, metalloproteases, and glycosidases, can shed light on the molecular changes directly related to the development of such diseases.[16,24,26]

The results found in the present study characterize the profile of GAG sulfates in HaCaT and A431 cell strains, and demonstrate the increase in HS and DS expressions in cell strains from SCC, as compared to those from keratinocytes, corroborating with data from prior literature, which show changes in such compounds during the development of cancer.

Curiously enough, prior literature has reported that the reduction of HA in biopsy samples from patients diagnosed with SCC is directly related to an unfavorable diagnosis of the disease, in turn suggesting that the reduction of HA immunomarkers presents a direct correlation with patients' lower survival rate.[27]

The reduction in the quantity of HA from cell fractions in A431 cells, when compared to HaCaT non-neoplasic cells, corroborates with the hypothesis that tumor cells with a lower cell differentiation present a lower quantity of HA.

HPSE is an endo-β-glucuronidase capable of cleaving HS strains of proteoglycans. The oligossacharides generated by the action of HPSE interact with greater affinity towards growth factors, angiogenic factors, and cytokines, thus intensifying the action of such molecules involved in cell processes, such as the development of tumors, proliferation, migration, cell invasion, and inflammatory response.

Tumor progression involves the degradation of extracellular matrix components, which clearly require the action of proteases and glycosidases.[28] The silencing of HPSE, using interference RNA (siRNA), demonstrated a significant reduction in the process of tumor metastasis and angiogenesis, indicating that such an enzyme is essential in the progress of molecular mechanisms of cancer development. Therefore, HPSE has become a potential target for antitumor therapy. [29]

Many reports in the literature prove that high levels of HPSE expression in mammal cells seem to be associated with the development of tumors and metastasis.[30,31]

The results obtained in the present study provide evidence that the levels of mRNA from the HPSE enzyme are increased in SCC, as compared to BCC, while non-neoplasic skin suggest a possible correlation of HPSE expression with skin cancer, which corroborates with that reported in prior literature.[18]

Treatment with HPSE inhibitors significantly reduces the incidence of tumor metastasis in trials that use the animal model, presenting high levels in advanced stages of the disease.[32,33]

The present study also found an expressive HPSE increase in BCC and SCC tumors, when compared to non-neoplasic tissues. These results demonstrate that the mRNA expression of the HPSE enzyme is significantly higher in nonmelanoma skin cancer. Such results contributed to the findings relative to HPSE expression in the A431 strain, which represents a cell strain formed in SCC.

According to the literature, the main cause of nonmelanoma skin cancer is one's exposure to ultraviolet radiation. Iriyama *et al.* reported that chronic exposure to UVB radiation activates the HPSE expression, in turn leading to the cleavage of the basal membrane's HS, resulting in changes in the epidermis and dermis of the skin that has been exposed to acute and chronic UVB radiation.[9] Kurdykowski *et al.* reported that mRNA expression and the enzymatic activity of HPSE were augmented depending on the dose of radiation used in studies with human keratinocyte cell cultures.[34]

The significant increase in HS in the A431 tumor strain may well suggest that such a compound can induce HPSE expression, since HS is a substrate of this enzyme, which will trigger the remodeling of the extracellular matrix in tumor tissues and will participate in carcinogenesis.

Prior literature defends that HPSE is involved in tumor angiogenesis and metastasis, suggesting that this enzyme is a promising target for the development of new therapies against nonmelanoma skin cancer.[35]

CONCLUSION

A significant difference can be observed between HPSE expression and the profile of GAGs when we analyze non-neoplasic human cell strains (HaCaT) and SCC strains (A431). The A431

strain, as compared to the HaCaT strain, presents a significant increase in HPSE expression. It can therefore be hypothesized that the increase in HPSE expression in the A431 strain may well be related to the increase in this strain's HS.

BCC and SCC samples present an increase in the mRNA expression of the HPSE enzyme, as compared to skin that has not been affected by such types of nonmelanoma cancer. Such results suggest that the HPSE is possibly linked to cell mechanisms involved in the development of BCC and SCC.

Future studies are warranted to elucidate the mechanisms of cell signaling involved in the increase of the HPSE enzyme expression in the development of BCC and SCC. ❑

REFERENCES

1. Inca.gov.br [Internet]. Tipos de Câncer. Pele não melanoma [acesso 21 Jul 2015] Disponível em: http://www.inca.gov.br/estimativa/2014/sintese-de-resultados-comentarios.asp.
2. Souza RJ, Mattedi AP, Corrêa MP, Rezende ML, Ferreira AC. Estimativa do custo do tratamento do câncer de pele tipo não-melanoma no Estado de São Paulo - Brasil. An Bras Dermatol. 2011;86:657-62.
3. Sampaio APS, Rivitti EA. Dermatologia. 3. ed. São Paulo: Artes Médicas; 2008.
4. Mantese SAO, Berbert ALCV, Gomides MDA, Rocha A. Carcinoma basocelular: Análise de 300 casos observados em Uberlândia MG. An Bras Dermatol. 2006;81:136-42.
5. Martinez MAR, Francisco G, Cabral LS, Ruiz IRG, Festa Neto C. Genética molecular aplicada ao câncer cutâneo não melanoma. An Bras Dermatol. 2006;81:405-19.
6. Favalli P, Bergonsi TO, Pavanello DP, Orsi V, Pase P, Schimidt M, et al. Carcinoma epidermóide de pele: Cutaneous epidermoid carcinoma. Revista da AMRIGS. 2007;51:301-5.
7. Wong CS, Strange RC, Lear JT. Basal cell carcinoma. BMJ. 2003;327:794-8.
8. Alam M, Ratner D. Cutaneous squamous-cell carcinoma. N Engl J Med. 2001;344:975-83.
9. Iriyama S, Matsunaga Y, Takahashi K, Matsuzaki K, Kumagai N, Amano S. Activation of heparanase by ultraviolet B irradiation leads to functional loss of basement membrane at the dermal-epidermal junction in human skin. Arch Dermatol Res. 2011;303:253-61.
10. Gallo R. Proteoglycans and cutaneous vascular defense and repair. J Investig Dermatol Symp Proc. 2000;5:55-60.
11. Malmström A, Bartolini B, Thelin MA, Pacheco B, Maccarana M. Iduronic acid in chondroitin/dermatan sulfate: biosynthesis and biological function. J Histochem Cytochem. 2012;60:916-25.
12. Zhao X, Yang B, Solakyildirim K, Joo EJ, Toida T, Higashi K, et.al. Sequence analysis and domain motifs in the porcine skin decorin glycosaminoglycan chain. J Biol Chem. 2013;288:9226-37.
13. Jeanloz RW. The nomenclature of mucopolysaccharides. Arthritis Rheum. 1960;3:233-7.
14. Gandhi NS1, Mancera RL. The structure of glycosaminoglycans and their interactions with proteins. Chem Biol Drug Des. 2008;72:455-82.
15. Schönherr E, Hausser HJ. Extracellular matrix and cytokines: a functional unit. Dev Immunol. 2000;7(2-4):89-101.
16. Theodoro TR, de Matos LL, Sant Anna AV, Fonseca FL, Semedo P, Martins LC, et. al. Heparanase expression in Circulating Lymphocytes of Breast Cancer Patients Depends on the Presence of the Primary Tumor and/or Systemic Metastasis. Neoplasia. 2007;9:504-10.
17. Edovitsky E, Lerner I, Zcharia E, Peretz T, Vlodavsky I, Elkin M. Role of endothelial heparanase in delayed-type hypersensitivity. Blood. 2006;107:3609-16.
18. Vlodavsky I, Friedmann Y. Molecular properties and involvement of heparanase in cancer metastasis and angiogenesis. J Clin Invest. 2001;108:341-7.
19. Jiang G, Zheng L, Pu J, Mei H, Zhao J, Huang K, et al. Small RNAs targeting Transcription Start Site Induce Heparanase Silencing through Interference with Transcription Initiation in Human Cancer Cells. PLoS One. 2012;7:e31379.
20. Komatsu N, Waki M, Sue M, Tokuda C, Kasaoka T, Nakajima M, et al. Heparanase expression in B16 melanoma cells and peripheral blood neutrophils before and after extravasation detected by novel anti-mouse heparanase monoclonal antibodies. J Immunol Methods. 2008;331:82-93.
21. Martins JR, Passerotti CC, Maciel RM, Sampaio LO, Dietrich CP, Nader HB. Practical determination of hyaluronan by a new noncompetitive fluorescence-based assay on serum of normal and cirrhotic patients. Anal Biochem. 2003;319:65-72.
22. Kozlowski EO, Pavao MS, Borsig L. Ascidian dermatan sulfates attenuate metastasis, inflammation and thrombosis by inhibition of P-selectin. J Thromb Haemost. 2011;9:1807-15.
23. Taylor KR, Rudisill JA, Gallo RL. Structural and sequence motifs in dermatan sulfate for promoting fibroblast growth factor-2 (FGF-2) and FGF-7 activity. J Biol Chem. 2005;280:5300-6.
24. Pecchi E, Priam S, Mladenovic Z, Gosset M, Saurel AS, Aguilar L, et.al. A potential role of chondroitin sulfate on bone in osteoarthritis: inhibition of prostaglandin E_2 and matrix metalloproteinases synthesis in interleukin-1β-stimulated osteoblasts. Osteoarthritis Cartilage. 2012;20:127-35.
25. Batista LT, Matos LL, Machado LR, Suarez ER, Theodoro TR, Martins JR, et.al. Heparanase expression and glycosaminoglycans profile in renal cell carcinoma. Int J Urol. 2012;19:1036-40.
26. Moscatello DK, Santra M, Mann DM, McQuillan DJ, Wong AJ, Iozzo RV. Decorin suppresses tumor cell growth by activating the epidermal growth factor receptor. J Clin Invest. 1998;101:406-12.
27. Kosunen A, Ropponen K, Kellokoski J, Pukkila M, Virtaniemi J, Valtonen H, et.al. Reduced expression of hyaluronan is a strong indicator of poor survival in oral squamous cell carcinoma. Oral Oncol. 2004;40:257-63.
28. Vlodavsky I, Korner G, Ishai-Michaeli R, Bashkin P, Bar-Shavit R, Fuks Z. Extracellular matrix-resident growth factors and enzymes: possible involvement in tumor metastasis and angiogenesis, in Cancer Metastasis. Cancer Metastasis Rev. 1990;9:203-26.
29. Edovitsky E, Elkin M, Zcharia E, Peretz T, Vlodavsky I. Heparanase gene silencing, tumor invasiveness, angiogenesis, and metastasis. J Natl Cancer Inst. 2004;96:1219-30.
30. Hulett MD, Freeman C, Hamdorf BJ, Baker RT, Harris MJ, Parish CR. Cloning of mammalian heparanase, an important enzyme in tumor invasion and metastasis. Nat Med. 1999;5:803-9.
31. Lerner I, Baraz L, Pikarsky E, Meirovitz A, Edovitsky E, Peretz T, et.al. Function of heparanase in prostate tumorigenesis: potential for therapy. Clin Cancer Res. 2008;14:668-76.
32. Li JP. Heparin, heparan sulfate and heparanase in cancer: remedy for metastasis? Anticancer Agents Med Chem. 2008;8:64-76.
33. Chen Y, Chen Y, Huang L, Yu J. Evaluation of heparanase and matrix metalloproteinase-9 in patients with cutaneous malignant melanoma. J Dermatol. 2012;39:339-43.
34. Kurdykowski S, Mine S, Bardey V, Danoux L, Jeanmaire C, Pauly G, et al. Ultraviolet-B irradiation induces epidermal up-regulation of heparanase expression and activity. J Photochem Photobiol B. 2012;106:107-12.
35. Elkin M, Ilan N, Ishai-Michaeli R, Friedmann Y, Papo O, Pecker I, et al. Heparanase as mediator of angiogenesis mode of action. FASEB J. 2001;15:1661-3.

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