10.1053.paor.2001.0304 available online at http://www.idealibrary.com on IDEAL

ARTICLE

Association of α_1 Acidic Glycoprotein and Squamous Cell Carcinoma of the Head and Neck

María Virginia CROCE,[1] Mike R PRICE,[2] Amada SEGAL-EIRAS[1]

[1]Centro de Investigaciones Inmunológicas Básicas y Aplicadas (CINIBA), Facultad de Ciencias Médicas, UNLP, La Plata, Argentina; [2]Cancer Research Laboratory, School of Pharmaceutical Sciences, University of Nottingham, UK

Serum from patients with different malignancies contain an abnormal concentration of α1-acidic-glycoprotein (AAG) and also, increased levels of AAG are associated with the presence of tumor mass. In the present report, serum levels of AAG were measured by radial immunodiffusion in squamous cell carcinoma of the head and neck (SCCHN) patients taking into account disease status parameters such as tumor localization, stage and extension of disease. Immunohistochemical methods, SDS-PAGE and Western-blotting were employed to study the expression of AAG and a carbohydrate related antigen (sialyl Lewis x) in tumor tissues and derived fractions. AAG showed abnormal levels in 7/15 oral cavity tumor patients' sera, 2/5 oropharynx and 5/10 larynx tumors; increased AAG serum levels belonged to patients with disseminated disease. On the other hand, the presence of AAG and sialyl Lewis x were demonstrated in carcinoma cells and in derived fractions from tumor tissues belonging to patients with elevated AAG serum levels. In the present study, we have found elevated levels of AAG in serum samples from SCCHN patients; these neoplastic cells are capable to express AAG. (Pathology Oncology Research Vol 7, No 2, 111–117, 2001)

Keywords: Head and neck cancer, α_1 acidic-glycoprotein, disease stage

Introduction

Head and neck tumors are common malignancies diagnosed at various stages of tumor progression and dissemination; approximately half of patients with cancer arising in the mucosa of the upper aerodigestive tract are cured of their initial tumor. The failure to achieve better therapeutic result should be attributed either to the advanced stage of disease at time of diagnosis or to insufficient effectiveness of treatment strategies, frequently as a consequence of the limited understanding of tumor biology as well as immunomodulatory molecular aspects on this type of tumors.

Serum from patients with different malignancies contains abnormal concentrations of acute phase proteins (APP) including squamous cell carcinoma of the head and neck (SCCHN).[2,19] These plasmatic proteins constitute a group of serum factors related to different immunological regulator functions and they have also been associated with tumor development and growth. In this sense, increased levels of α_1 acidic glycoprotein (AAG) have been reported in patients with different malignant diseases,[28,29] it has been also detected in association with specific antibodies in serum samples belonging to patients with advanced esophagous cancer.[3]

Furthermore, AAG can behave as an immunomodulatory agent inducing a marked inhibition on the proliferative response of human peripheral blood lymphocytes.[1,6] In inflammatory conditions, de Graaf et al[8] have reported the expression of sialyl-Lewis x in AAG and Dage et al[7] developed a sensitive method to verify the presence of sialyl Lewis x on AAG molecule; this antigen is frequently expressed on neoplastic cells and it has been strongly related to cellular migration and metastatic process.[25]

With regard to carcinoma cell expression of acute phase proteins, there is evidence[5,12] suggesting that neoplastic cells are able to synthesize these reactants. While increased levels of AAG are associated with the presence

Received: Jan 15, 2001; *accepted:* April 10, 2001

Correspondence: Prof Amada SEGAL-EIRAS, CINIBA, Facultad de Ciencias Médicas, Calle 60 y 120, 1900, La Plata, Argentina. Fax 0054 221 4871481; E-mail: as-eiras@netverk.com.ar

 1219-4956/01/020111+07 $ 12.00/0

of tumor mass, the hepatic and/or tumor source of this AAG is less clear; furthermore, its biological and/or immunological roles have not been yet clarified[27] although there is evidence that it may behave as an immunosuppressive factor in cancer.[9]

The present study was performed on serum and tumor samples belonging to advanced SCCHN patients with the purpose of measure AAG serum levels and to investigate whether their tumor tissues express this molecule as well as a related carbohydrate antigen, the sialyl Lewis x.

Materials and Methods

Serum and tumor tissue samples

Serum and tumor samples were selected from patients at the Department of Surgery, University Hospital, La Plata, Argentina. The written consent of patients and controls involved in this research was obtained before beginning the study, according to the Convention for the Protection of Human Rights and Dignity of the Human Beings with regard to the application of Biology and Medicine of Europe, Strasbourg, 1996.

The project was approved by the Ethical Committee of the University Hospital.

Criteria for patient inclusion in this study were as follows: 1) a histologically confirmed diagnosis of SCCHN of the upper aerodigestive tract, 2) absence of history of any other tumor localization such as non-head and neck squamous cell carcinoma and 3) absence of clinical local or systemic infection. Therefore, patients with a previous malignancy (SCCHN or non-SCCHN), treated with anti cancer therapy and individuals with local infection, sepsis, immunosuppression and autoimmune disease were excluded from this study.

Tissue and serum samples from previously untreated patients with primary SCCHN were studied from 30 patients (28 men and 2 women); mean age at diagnosis was 63.7 years (range, 40-80 years). Data of tumor localization were as follows: oral cavity (n=15), larynx (n=10) and oropharynx (n=5). All tumours were classified according to UICC criteria TNM Classification of Malignant Tumors, 5th edition.[15] One patient had a tumor stage I; another one, stage II while 24 presented stages III and IV while 4 remained undetermined; at time of diagnosis, 40% of patients had nodular metastasis and only three presented pulmonary metastasis; patients data are shown in *Table 1*.

Tumor and serum samples from 7 patients with colorectal cancer (Dukes C stage), age and sex matched were included as positive controls. AAG median serum level was 170 mg/dl (range, 135-204) and all tumor tissue samples expressed AAG by immunohistochemical methods.

A group of 20 healthy age and sex matched donors were included as controls. Venous blood was obtained aseptically from patients and controls, allowed to clot at room temperature and centrifuged at 3000 x g at 4°C; sera were separated, aliquoted and frozen at –80°C. Samples were thawed out only once before used.

Normal squamous epithelial tissues were obtained by biopsy at the same localization and included as controls.

AAG detection

Serum AAG was measured by radial immunodiffusion using DIFFU-PLATE plates (Biocientífica, Argentina). AAG values in a range from 43 to130 mg/dl were considered as normal.

Preparation of extranuclear membrane fractions

Fractions were prepared from human tumor tissues.[26] Briefly, tissues were homogeneized in 1.41 M PBS, pH 7.2, at 4 ml/g; homogenates were centrifuged at 600 x g and at 105000 x g at 4°C and precipitates containing extranuclear membrane fractions were resuspended in 1.41M PBS, liophylized and stored at –20°C for subsequent density gradient centrifugation.

SDS-PAGE and immunoblotting

Fractions were collected and dialysed against 1.41 M PBS at 4°C for 48 h and then liophylised, resuspended in SDS-PAGE sample buffer at reducing conditions and run following standard procedures[20] in a discontinuous buffer system. After electrophoresis gels were either stained with Coomassie blue or were transferred electrophoretically to nitrocellulose membranes[32] and incubated with different monoclonal antibodies.

Immunohistochemical analysis

This technique was developed according to a previous report,[4] with minor modifications. All specimens were fixed in phosphate buffered formalin, embedded in paraffin and cut into 5 μm serial sections. Deparaffinized sections were treated with 10 mM sodium citrate buffer at 100°C for 5 minutes,[3] then, they were incubated overnight at 4°C with mouse monoclonal antibodies. Controls were incubated with PBS instead of monoclonal antibodies. The whole area of each sample was observed by sequentially examining low power (x10) optical fields and with higher magnifications (x40, x63, x100); the staining of cytoplasm, plasma and nuclear membranes were also evaluated. Cells were considered as positive when at least one of these components was stained; the heterogeneous reactivity was graded according to the positive reaction, intensity and distribution. Staining intensity was graded as negative, low, moderate and strong.[10]

Antibodies

Monoclonal antibody KM93 (IgM), an anti sialyl-Lewis x^{14} and a rabbit anti-human orosomucoid (α_1 acidic glycoprotein) (Code No. A011, DAKO, USA) were assayed for immunohistochemical analysis as well as immunoblotting assays.

Statistical analysis

Normal distribution and homoscedasticity of data were studied by Kolmogorov test and Bartlett methods, respectively. Each variable was analyzed by ANOVA and multiple comparisons among groups were performed by Tukey (HSD) test ($p<0.05$).[34] Spearman and Kendall rank correlation was run among variables.

***Table 1.* Summary of clinicopathological features and AAG levels in pretreatment head and neck cancer patients.**

Case number	*Tumor localization*	*TNM*	*Stage*	*AAG*
1	OC	T1N0M0	I	33,3
2	OC	T2N0M0	II	75,1
3	OC	T3N0M0	III	58
4	OC	T3N0M0	III	42,4
5	OC	T2N3M0	IVc	135,5
6	OC	T3N3M0	IVb	113,8
7	OC	T3N0M1	IVc	75,1
8	OC	T2N3M0	IVb	101,5
9	OC	T2N2M0	IVa	214,3
10	OC	T4N2M0	IVb	93,7
11	OC	T3N2M0	IVa	214,3
12	OC	T3N2M1	IVb	182,9
13	OC	ND	ND	93,2
14	OC	T3N2M0	IVa	182,9
15	OC	ND	ND	28,3
16	OP	T3N3M0	IVb	113,8
17	OP	T4N0M0	IVa	208,9
18	OP	T3N3M0	IVb	93,7
19	OP	T3N3M0	IVb	214,3
20	OP	ND	ND	42,4
21	LA	T3N1M0	III	158,4
22	LA	T3N0M0	III	75,1
23	LA	T4N0M0	IVa	135,5
24	LA	T4N0M0	IVa	135,3
25	LA	T4N0M0	IVa	75,1
26	LA	T4N1M0	IVb	114
27	LA	T4N2M0	IVb	113,8
28	LA	T3N0M1	IVc	158,4
29	LA	T4N2M0	IVb	154
30	LA	ND	ND	93,7

ND: not determined. AAG: α-1-Acidic Glycoprotein; OC: oral cavity; OP: oropharynx; LA: larynx. – AAG levels are expressed in mg/dl.

Results

Serum measurements

Table 1 shows results obtained in pretreatment serum samples from all SCCHN tumor localizations included in this investigation.

AAG measurement demonstrated that 7 out of 15 (46%) patients with oral cavity tumors presented abnormal values, two patients had AAG values below normal and one near the lower limit (patient no.4, 42,4 mg/dl) (*Table 1*) while five patients showed serum levels above normal. In oropharynx localization, 2 out of 5 patients presented increased levels while another serum showed AAG values near the lower limit; on the other hand, in larynx located tumors, values were elevated in 50% (5/10).

No statistical differences in AAG serum levels were observed when tumor localization was considered (*Figure 1*); however, AAG levels showed an increasing tendency according with tumor stage; AAG was about 33,3 mg/dl in the patient of stage I and 75 mg/dl in the patient at stage II; AAG median levels yielded 83,5 in stage III (range 42.4-158.4) and 141,5 in stage IV (range 75-214), (normal values vary from 43 to 130 mg/dl). Furthermore, a high proportion of patients (9/12, 75%) showing increased levels of AAG had node involvement and/or pulmonar metastatic dissemination, while the other 3 patients presented tumors identified as T4 (cases no.17, 23 and 24; *Table 1*).

Samples from 20 healthy controls revealed a mean level of 75 mg/dl (SD=26). Positive controls were also included as it has been mentioned in Materials and Methods.

Tissue analysis

By routine techniques, histopathological examination was performed; all head and neck tumors were diagnosed as invasive squamous cell carcinoma.

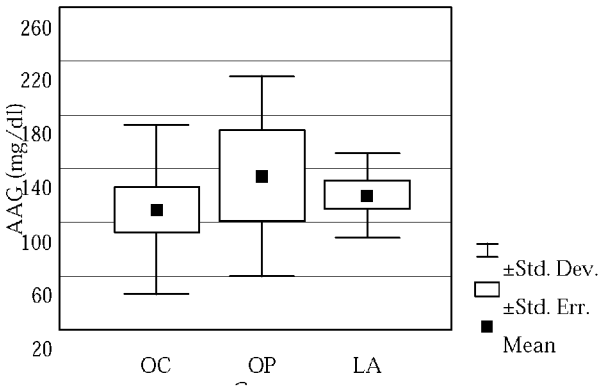

Figure 1. *AAG levels (mg/dl) according to tumor localization. ANOVA test with Tukey analysis was performed; no difference among groups was detected. OC: oral cavity; OP: oropharynx and LA: larynx.*

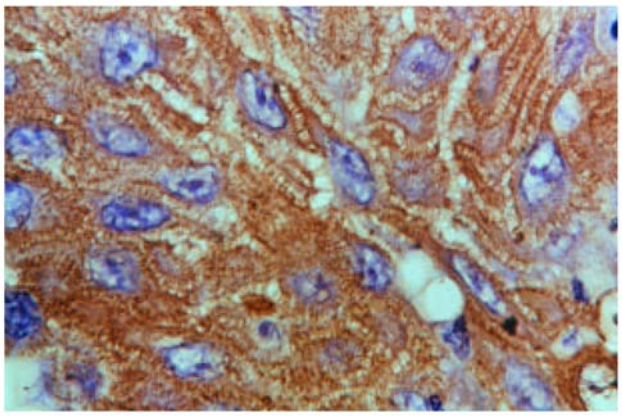

Figure 2a. *Immunohistochemical analysis in an oropharynx squamous cell carcinoma incubated with anti-AAG antibody; this area shows cytoplasm staining with a discontinuous pattern at the plasma membrane and intercellular bridges (x63).*

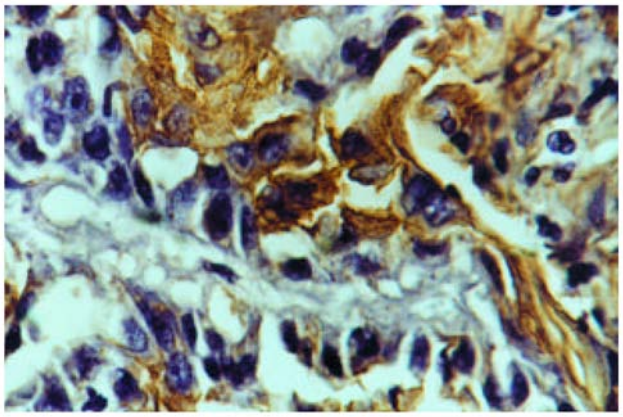

Figure 2b. *Head and neck with anti sialyl Lewis xMAb (KM93); some cells show a positive reaction at the cytoplasm and plasma membrane (x63).*

In order to study AAG expression in tumor cells, immunohistochemical studies were done; when anti-AAG antibody was assayed, 40% of the tumors showed a positive reaction in some cells with a homogeneous cytoplasmic staining while with a discontinuous pattern in nuclear and plasma membrane; the common feature observed was a strong reaction in some areas of the tumor mass (*Figure 2a*) while some other parts remained negative. Sialyl Lewis x–containing structures have been described as a part of AAG; to determine whether SCCHN cells express sialyl Lewis x, KM93 (anti-sialyl Lewis x MAb) was employed; in several tumors, reactivity was strong in cellular membranes of several cells including cytoplasmic staining in some of them (*Figure 2b).*

In normal squamous epithelium belonging to the same localization, AAG expression was observed restricted to some cells at different layers with a diffuse and moderately cytoplasmic pattern (*Figure 3*) while sialyl Lewis x was expressed in a few basal lining cells.

A group of 8 colorectal adenocarcinoma reacting positively with AAG as well as with sialyl Lewis x were employed as positive controls. The AAG reaction of some samples consisted in a strong and diffuse staining mostly at cytoplasmic level (*Figure 4a*); some other tumor samples showed a different pattern since membranes were mainly reactive.

Anti sialyl Lewis x MAb showed well-defined areas with a moderate or weak staining; it was possible to observe the distribution of some positive cells in several malignant glands which reacted either at the cytoplasm and/or at the apical cell membrane *(Figure 4b);* debris showed also a positive immune reaction.

In some cases, it was possible to examine both the primary tumor and the metastatic regional node; in two laryngeal carcinomas, the primary tumor was stained positively with anti AAG (*Figure 5a*) and with anti sialyl-Lewis x while in lymph node, neoplastic cells reacted only with anti-AAG (*Figure 5b*).

In colorectal carcinoma, a primary tumor and a metastatic node from the same patient reacted strongly with anti-AAG and with anti-sialyl Lewis x (data not shown).

The immune reaction obtained with the antibodies assayed here were significantly higher in advanced stages compared with earlier counterparts of these malignant diseases.

SDS-PAGE and Western-blotting analysis

Extranuclear membranes were prepared and analysed by SDS-PAGE and Western-blotting (WB) employing anti-AAG antibody. *Figure 6* shows this reaction in a sample belonging to a larynx carcinoma; a smear pattern of reactivity was obtained with several bands of low molecular weight that clearly show the presence of AAG.

ENM fractions belonging to different head and neck tumor localizations showed a positive reaction when anti-sialyl Lewis x MAb was tested.

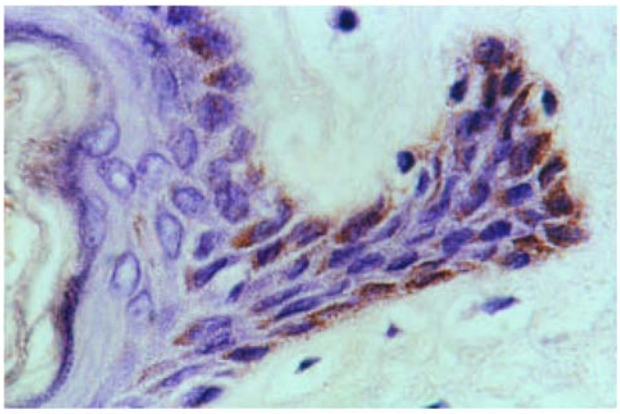

Figure 3. *Normal oral tissue incubated with anti-AAG antibody. A mild perinuclear reaction with a granular pattern is observed mainly in basal cells (x63).*

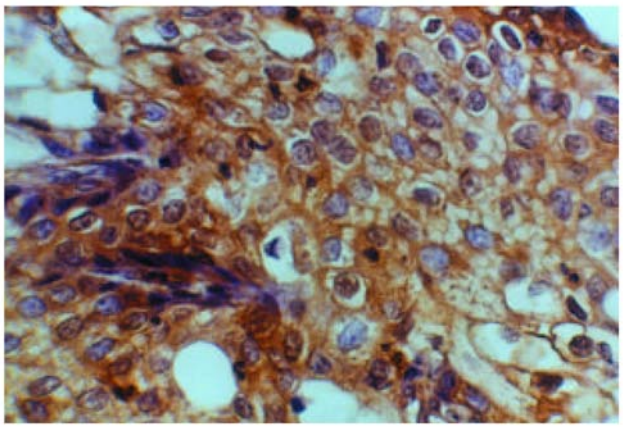

Figure 4a. *Colorectal tumor section incubated with anti AAG antibody. The positive reaction is observed at the plasma membrane, on the left of the picture a cytoplasmic staining is seen (x63).*

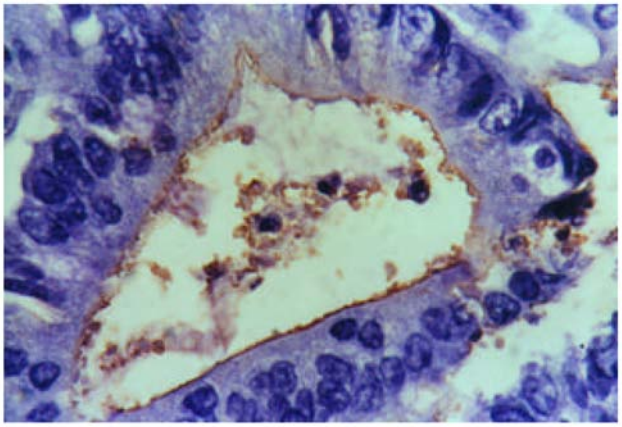

Figure 4b. *Immunohistochemistry of a colorectal adenocarcinoma incubated with KM93 MAb (anti sialyl Lewis x). A positive reaction at the apical plasma membrane and cellular debris is found; a superficial droplets of mucosubstance are observed (x63).*

Fractions belonging to colorectal tumors showed WB similar results while normal tissues of head and neck localization did not express neither AAG nor sialyl Lewis x (data not shown).

Discussion

APP are not tumor specific since the acute phase response is non-specific and can be induced by any change in homeostasis. APP show usually abnormal levels during infections, neoplasia, autoimmune diseases and also in stressed or traumatic conditions; they depend on the cytokine network modulation. AAG is considered one of the major APP; the gene encoding this protein is positively controlled at the transcriptional level by cytokines such as IL-1, IL-6 and tumor necrosis factor alpha as well as by glucocorticoids. Human hepatocytes are normally the site of AAG production but it is uncertain whether AAG serum levels increase as a response of the host to tumor growth or as a consequence of neoplastic cell production. It has been found that cells from SCCHN[21] and some other tumors[33] can synthesize different interleukins including IL-6 as well as some APP such as AAG.

Our study has revealed that patients with advanced tumor stages present increased levels of AAG; other authors[2] have obtained similar results in head and neck cancer; furthermore, in the same tumor localization, Suárez-Nieto et al[30] have found that only AAG values greater than 150 mg/dl (compared with haptoglobin, α_1-antitrypsin, α_2-H5 glycoprotein and prealbumin) were a prognostic factor of a similar precision to those of tumor stage.

We have obtained by immunohistochemistry an interesting result such as the detection of this APP in tumor tissue sections in coincidence with its isolation from tumor fractions. The SDS-PAGE and Western blotting of ENM prepared from tumor tissues from SCCNH and colorectal can-

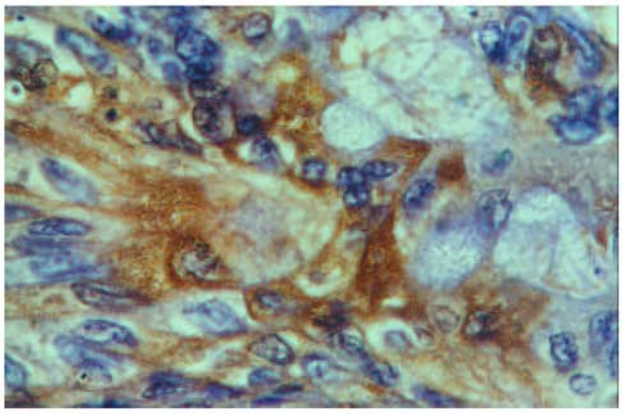

Figure 5a. *Immunohistochemical staining of a larynx squamous cell carcinoma with anti-AAG antibody. A positive granular pattern staining is observed in some cells while in others it is negative (x63).*

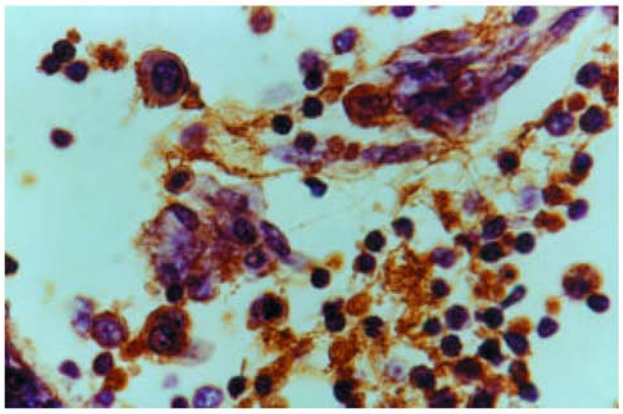

Figure 5b. *Immunohistochemistry on a lymphatic node section belonging to the same patient of Figure 5a incubated with AAG antibody. Some sinus located metastatic cells showed a cytoplasmic and plasma membrane staining (x63).*

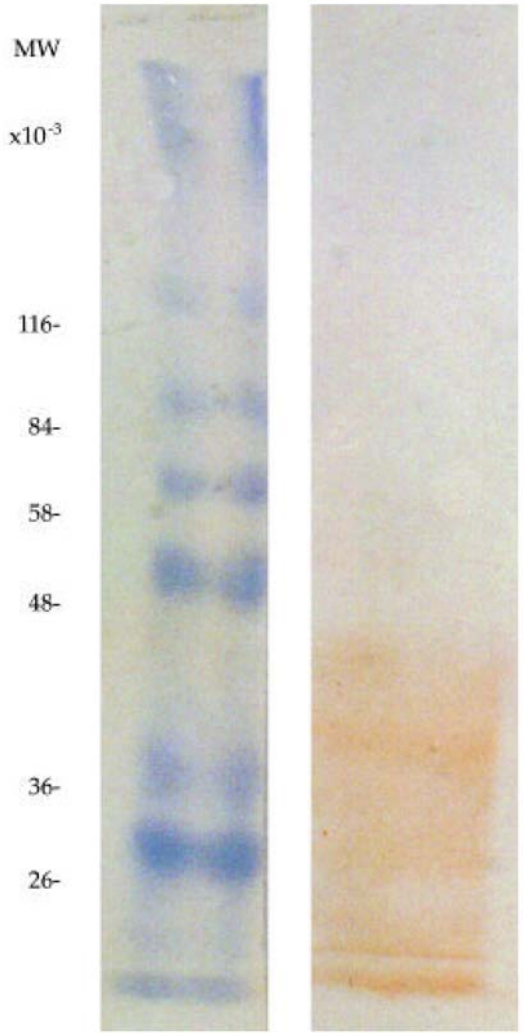

Figure 6. *SDS PAGE and Western-blotting of subcellular fractions derived from a larynx tumor. After incubation with anti-AAG antibody; a positive reaction with a smear pattern at low molecular weight (less than 45kD) was obtained.*

cer showed a smear pattern at low molecular weight. In AAG derived from serum samples, bands of low molecular weight were identified,[25] the presence of several bands are coincident with AAG molecular structure since different chains have been previously described.[5]

On the other hand, another goal of our research has been AAG expression associated with sialyl Lewis x reactivity in some samples of SCCHN and in colorectal cancer samples as positive controls. These observations are consistent with previous findings since it is known that sialyl Lewis x is predominantly expressed in digestive tract cancers.[18] It is interesting to point out that cells belonging to normal tissues may express these antigens which agrees with results obtained by most investigators, taking into account that tumor associated antigens are not exclusively expressed by neoplastic cells; usually there is either an increase or a different pattern of expression observed in neoplastic versus normal cells.

Tumor cells can gain either lymphatic or blood vessels and adhere to their walls through the expression of carbohydrate chains which facilitate cancer cell migration; several authors[16,24] have described the expression of sialyl Lewis x and sialyl Lewis a in association with invasion and metastases in different neoplasias. Moreover, it has been demonstrated the expression of sialyl-Lewis a in SCCHN both in tumor sections as well as in tumor cell lines.[17,22] The presence of these carbohydrate ligands have been described in AAG molecules,[8] this is also consistent with the association of AAG with tumor spreading. However, it has been reported that mucins as well as some other glycoproteins and glycolipids may behave as possible sialyl Lewis x carriers.[11,23] Hakomori et al[13] and other authors have considered that different glycans may be involved in tumor spreading and also they may participate in diverse molecule structures.

This report shows that a high proportion of patients presents AAG abnormal levels; taking into account that most patients showed advanced tumor stages, AAG values may be associated with progression of disease. We have also observed that there was no apparent correlation between AAG increased levels and tumor localization; this finding should be expected in markers related to carcinoma growth and progression, since they may share a similar function regardless the site of tumor origin.

The identification of a common denominator in different types of cancer despite their localization but with a coincident stage of dissemination, may provide the means to enhance biological understanding and knowledge of clinical relevance in these neoplastic conditions. Further investigations of AAG and its relation to malignancy may delineate a possible indirect control that it exerts in many aspects of SCCHN growth and progression.

Acknowledgements

Authors are thankful to Dr. C. Pereyra and co-workers for providing patients samples and to Lic. S.O. Demichelis for her help in statistical analysis. Financial support obtained from the CICPBA (Comisión de Investigaciones Científicas de la Pcia. de Buenos Aires) and University of La Plata and CONICENT is very appreciated.

References

1.[2] *Bennett M, Schmid K:* Immunosuppression by human plasma α_1 acid glycoprotein: importance of the carbohydrate moiety. Proc Natl Acad Sci USA 77:6109-6114, 1980.

2.[2] *Caldani C, Thyss A, Schneider M, et al:* Orosomucoid: prealbumin ratio- a marker of the host-tumor relationship in head and neck cancer. Eur J Cancer Clin Oncol 24:653-657, 1988.

3.[2] *Croce MV Segal-Eiras A:* Identification of acute-phase proteins (APP) in circulating immune complexes (CIC) in esophageal cancer patients' sera. Cancer Invest 4:421-426, 1997.

4. *Croce MV, Colussi AG, Price M, et al:* An immunohistopathological characterization of spontaneous metastases in a human lung mucoepidermoid adenocarcinoma (HLMC) xenograft. Pathol Oncol Res 4:259-266, 1998.
5. *Chandrasekaran EV, Davila M, Nixon D, et al:* Structures of oligosaccharide chains of two forms of α_1 acidic glycoprotein purified from liver metastases of lung, colon and breast tumors. Cancer Res 44:1557-1567, 1984.
6. *Cheresh DA, Haynes D, Distasio JA:* Interaction of an acute phase reactant α_1 acid glycoprotein [orosomucoid] with the lymphoid cell surface a model for non-specific immune suppression. Immuno 51:541-547, 1984
7. *Dage JL, Ackermann BL, Halsall HB:* Site localization of sialyl Lewis^x antigen on α_1 acid glycoprotein by high performance liquid chromatography-electrospray mass spectometry. Glycobiol 8:755-760, 1998.
8. *De Graaf TW Van der Stelt ME, Anbergen MG:* Inflammation induced expression of sialyl-Lewis x-containing glycan structures on alpha 1 acidic glycoprotein (orosomucoid) in human sera. J Exp Med 177:657-666, 1993.
9. *Elg SA, Mayer AR, Carson LF, et al:* α_1-acidic glycoprotein is an immunosuppressive factor found in ascites from ovarian carcinoma. Cancer 80:1448-1456, 1997.
10. *Feickert HJ, Anger BR, Cordon-Cardo C, et al:* Cell-surface antigens of human lung tumours detected by mouse monoclonal antibodies: definition of blood-group- and non-blood-group-related antigenic systems. Int J Cancer 46:1007-1013, 1990.
11. *Feizi T:* Demonstration by monoclonal antibodies that carbohydrate structures of glycoproteins and glycolipids are oncodevelopmental antigens. Nature 314:53-57, 1985.
12. *Fujii M, Takahashi N, Hayashi H, et al:* Comparative study of alpha1-acidic glycoprotein molecular variants in ascitic fluid of cancer and non-cancer patients. Anticancer Res 8:303-306, 1988.
13. *Hakomori S, Nudelman E, Levery SB:* Novel fucolipids accumulating in human adenocarcinoma: I. Glycolipids with di- or trifucosylated type 2 chain. J Biol Chem 259:4672-4680, 1984.
14. *Hanai N, Shitara K, Yoshida H:* Generation of monoclonal antibodies against human lung squamous cell carcinoma and adenocarcinoma using mice rendered tolerant to normal human lung. Cancer Res 46:4438-4443, 1986.
15. International Union Against Cancer (UICC) Classification of Malignant Tumours. Ed. LH Sobin and Ch Wittekind, 5th edition, New York, 1997.
16. *Irimura T, McIsaac AM, Matsushita Y, et al:* Colorectal cancer metastasis determined by carbohydrate–mediated cell adhesion: role of sialyl-Le^x antigens. Semin Cancer Biol 4:319-324, 1993.
17. *Itoh T, Yonezawa S, Nomoto M, et al:* Expression of mucin antigens and Lewis x related antigens in carcinomas and dysplasia of the pharynx and larynx. *Pathol Int* 46:646-655, 1996.
18. *Kannagi R:* Carbohydrate-mediated cell adhesion involved in hematogenous metastasis of cancer. *Glycoconjugate J* 14:577-584, 1997.
19. *Krecicki T, Leluk M:* Acute phase reactant proteins-an aid to monitoring surgical treatment of laryngeal carcinoma. J Laryngol Otol 106:613-619, 1992.
20. *Laemmli UK:* Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (Lond) 227:680-681, 1980.
21. *Ljungberg B, Grankvist K, Rasmuson T:* Serum interleukin-6 in relation to acute-phase reactants and survival in patients with renal cell carcinoma. Eur J Cancer 33:1794-1798, 1998.
22. *Makino K, Ogata T, Miyake H, et al:* Expression of tumor associated glycoantigen, sialyl Lewis a, in human head and neck squamous cell carcinoma and its application to tumor immunotherapy. Jpn J Cancer Res 85:887-891, 1994.
23. *Matsushita Y, Cleary KR, Ota DM, et al:* Sialyl-dimeric Lewis-X antigen expressed on mucin-like glycoproteins in colorectal cancer metastases. Lab Invest 63:780-790, 1990.
24. *Nakamori S, Kameyama M, Imaoka S, et al:* Involvement of carbohydrate antigen sialyl Lewis (x) in colorectal cancer metastasis. Dis Col Rectum 40:420-431, 1997.
25. *Phillips ML, Nuddelman E, Gaeta FCA, et al:* ELAM-1 mediates cell adhesion by recognition of a carbohydrate ligand, Sialyl-Lex. Science 250:1130-1132, 1990.
26. *Price MR, Edwards S, Owainati A, et al:* Multiple epitopes on a human breast carcinoma associated antigen. Int J Cancer 36:567-574, 1985.
27. *Schultz D, Arnold P:* Properties of four acute phase proteins: C-reactive protein, serum amyloid A protein, α_1-acidic glycoprotein and fibrinogen. Sem Arthr Rheum 20:129-147, 1990.
28. *Stamatidis A, Toumanidou M, Vyssoulis G:* Value of serum acute-phase proteins and CEA in preoperative staging of colorectal cancer. Cancer 65:2055-2057, 1990.
29. *Stanciu L, Dumitrascu D, Radu D:* Non-specific tumoral markers in hepato-cellular carcinoma. Med Intern: 28:139-144, 1990.
30. *Suárez-Nieto C, Cuesta-García A, Fernández-Bustillo E, et al:* Serum glycoproteins and prognosis in cancer of the head and neck. Clin Otolaryngol 11:41-45, 1986.
31. *Switacheva N, Davies JR, Lesuffleur T:* Characterization of mucins produced by human HT29-MTX cell line. 5th International Workshop on Carcinoma Associated Mucins Cambridge, UK, 1998.
32. *Towbin H, Staehelin T, Gordon J:* Electrophoretic transfer of proteins from polyacrilamide gels to nitrocellulose sheets: procedures and some applications. Proc Natl Acad Sci USA 76:4350-4354, 1979.
33. *Woods KV, El Naggar A, Clayman GL, et al:* Variable expression of cytokines in human head and neck squamous cell carcinoma cell lines and consistent expression in surgical specimens. Cancer Res 58:3132-3141, 1998.
34. *Zar JH:* Biostatistical Analysis, 3rd ed, Prentice Hall, New Jersey, USA, 1996.