RESEARCH ARTICLE

Is Immunohistochemical Sex Hormone Binding Globulin Expression Important in the Differential Diagnosis of Adenocarcinomas?

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Abstract

Adenocarcinomas (AC) are the most frequently encountered carcinomas. It may be quite challenging to detect the primary origin when those carcinomas metastasize and the first finding is a metastatic tumor. This study evaluated the role of sex hormone binding globulin (SHBG) positivity in tumor cells in the subclassification and detection of the original organ of adenocarcinomas. Between 1994 and 2008, 64 sections of normal tissue belonging to ten organs, and 116 cases diagnosed as adenoid cystic carcinoma and mucoepidermoid carcinoma of the salivary gland, lung adenocarcinoma, invasive ductal carcinoma of the breast, adenocarcinoma of stomach, colon, gallbladder, pancreas and prostate, endometrial adenocarcinoma and serous adenocarcinoma and mucinous adenocarcinoma of the ovary, were sent to the laboratory at the Department of Pathology at the Yuzuncu Yil University School of Medicine, where they were stained immunohistochemically, using antibodies against SHBG. The SHBG immunoreactivity in both the tumor cells and normal cells, together with the type, diffuseness and intensity of the staining were then evaluated. In the differential diagnosis of the adenocarcinomas of the organs, including the glandular structures, impressively valuable results are encountered in the tumor cells, whether the SHBG immunopositivity is evaluated alone or together with other IHC markers. Further extensive research with a larger number of cases, including instances of cholangiocarcinoma and cervix uteri AC [which we could not include in the study for technical reasons] should be performed, in order to appropriately evaluate the role of SHBG in the differential diagnosis of AC.

Keywords: SHGB - adenocarcinoma - differential diagnosis

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Introduction

Adenocarcinoma (AC) is a malignant tumor of the epithelium gland. It includes malignant tumors of the stomach, intestinal mucosa, pancreas, gallbladder, liver, breast, prostate, endometrium, cervix, ovary, lung and salivary glands. Many immunohistochemical markers have been used in the differential diagnosis of ACs. However, there are still pathological diagnostic problems in detecting the primary tumor and especially in poorly differentiated carcinoma metastases.

Human sex hormone binding globulin (SHBG) is a glycoprotein of 85,000 daltons in a homodimeric structure. Each monomer includes a region of steroid binding (Grishkovskaya et al., 2000; Avvakumov et al., 2001). It is a major plasma protein, which provides transport for the biologically active sex steroids and plasma concentration of SHBG, and affects the quantity, half-life and transport of the free sex steroids in the circulation to target tissues (Westphal, 1986; Hammond, 1995). SHBG, a 373-amino-acid glycoprotein, previously known as a carrier protein, is classically understood to be synthesized mainly in the liver and then secreted into the circulating system (Maliqueo et al., 2007; Bobe et al., 2008; Fortunati et al., 2010). It binds to sex steroids in the circulation with a high affinity, modulating the bioavailability of the hormon es such as estrogen, androgen and testosterone (Mahlck et al., 1986; Selby et al., 1990; Garcia-Closas et al., 2007). It is also expressed in several other tissues such as those of the brain (Joseph et al., 1991; Herbert et al., 2005), uterus (Misao et al., 1995), testis (Hagen et al., 1992; Selva et al., 2006), breast (Kahn et al., 2008), ovary (Forges et al., 2004) and placenta (Larrea et al., 1993), and prostate (Hrby et al., 2002; Kahn et al., 2008), which are classic target tissues for androgens and estrogens (Mahlck et al., 1989; Misao et al., 1995; Langdon et al., 2009).

In this study we aimed to define whether there is a place for SHBG expression in the differential diagnosis of ACs originating in different organs. For this reason, we conducted SHBG immunohistochemistry staining in normal tissue samples of 10 organs and in AC samples from 12 tissue sites.

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Materials and Methods

Study subject

One hundred and sixteen cases diagnosed as: adenoid cystic carcinoma (ACC) and mucoepidermoid carcinoma (MEC) of the salivary gland, lung adenocarcinoma (LAC), invasive ductal carcinoma of the breast (IDC), adenocarcinoma of stomach (SAC), colon (CAC), gallbladder AC (GBAC), pancreas AC (PAC), prostate AC (PRAC), endometrial adenocarcinoma (EAC) and serous adenocarcinoma (OSAC) and mucinous adenocarcinoma (OMAC) of the ovary, together with five to six normal tissue samples of all the organs and 12 normal tissue samples of endometrium (six proliferative, six secretory phase endometrium) sent to the Laboratory of the Department of Pathology at the Yuzuncu Yil University School of Medicine between 1994 and 2008, were selected.

Preparations of the cases were brought from the lab archives, re-evaluated and appropriate blocks were selected for imunohistochemical staining. Cervix and liver cases were excluded, since cervix AC and cholangiocarcinomas were not present in the archives.

Immunohistochemical study

All sections of the cases stained with Hematoxylin Eosin (H&E) were re-evaluated using light microscopy. H&E stained paraffin blocks, one for each tumor thought to be the best sample of that tumor, were selected for immunohistochemical staining with SHBG.

Cross-sections with a thickness of four microns were deparaffinized in xylene, dehydrated in ethanol series, and incubated for 10 minutes in 3% H_2O_2.

Afterwards, the glass slide was rinsed thor¬oughly with distilled water for 20 minutes and exposed to antigen for a total period of 20 min¬utes, with a one-minute break at five-minute intervals, in a target retrieval solution diluted by 1/10. The solution was left for twenty minutes at room temperature and then washed with distilled water. After that it maintained for five minutes in phosphate-buffered saline (PBS). Next, it was incubated for one hour with 1/200 diluted SHBG antibody. Prof. Dr. Catherine Grenot, a faculty member at RERM laboratories, provided the SHBG antibody used in this study. Then, it was kept for 10 minutes in PBS. After it was incubated for approximately 15 minutes in a biotin solution, it was kept for 10 minutes in PBS. Then, it was incubated for about 15 minutes in a streptavidin peroxidase solution. It was kept for five minutes in AEC (3 amino-9-etylcarbazole) chromogen after it was kept in PBS for 10 minutes. It was washed with distilled water. The cross-sections were then kept for five minutes in Mayer's hematoxylin for counter-staining. Afterwards, it was rinsed with tap water and encased in a mounting medium (Entellan, Merck Millipore, Darmstadt, Germany). The preparations stained with SHBG were evaluated according to the type, extensiveness and intensity of the staining cells. They were evaluated as cytoplasmic, membranous and secretory staining, according to the type of the staining.

The staining intensity was assessed semi-qualitatively as 0, referring to no staining (negative staining) (score 0); +/-, if a weak positivity was observed (score 1); + if a mild, positive staining was observed (score 2); ++ if a moderate staining intensity was observed (score 3); +++ if an extremely strong staining intensity (score 4) was observed.

According to the diffuseness of the staining, sections were graded as: 0, no staining; 1, staining <25%; 2, staining between 25% and 50%; 3, staining between 50% and 75%; or 4, staining >75%.

Statistical evaluation

The descriptive statistics of the staining properties of the carcinomas on each organ were expressed as a number and percentage. A Chi-square test was conducted to define whether these properties had any association with the organ and organ regions. Also, a Simple Coherence Analysis was carried out to visually define the associations between these parameters. The statistical level of significance was established at 5% and all calculations were made using SPSS (Statistical Package for Social Sciences) 13.0 for Windows® statistical package program.

Results

The normal and tumoral cases stained with SHBG were evaluated according to the type of staining, intensity and diffuseness of the staining. In the staining of normal tissues of the related organs no staining was observed in the salivary gland, gallbladder and colon. Secretory staining was positive in all tissue samples of lung and prostate at (++) intensity and diffuseness. In three patients out of six with secretory endometrium, (+) staining was observed. In one patient out of six with secretory endometrium, (++) staining was observed. In four patients out of six with proliferative endometrium, (+) staining was observed. In three patients out of six with normal stomach tissue, (+++) intensity and diffusenerss staining was observed cytoplasmic staining . Out of the six ovarian tissue samples, cytoplasmic staining was present at (+++) intensity in the granulosa cells in three cases and secretory staining in (++) intensity in one case. No

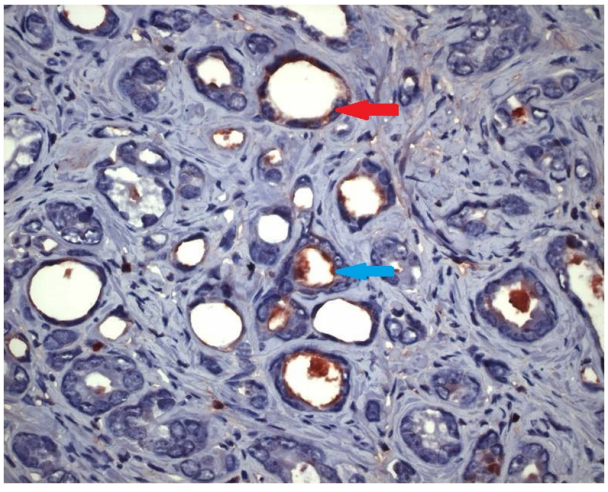

Figure 1. (++) Cytoplasmic staining (red arrow) with SHBG in prostate AC and secretory staining (blue arrow) (Immunoperoxidase x 400).

granulosa tissue was observed in one ovarian tissue and no staining was seen in the ovarian stroma in that tissue, either. Cytoplasmic staining was observed in two in (++) intensity, in two in (+) among six breast tissues; and (+++), (++) and (+) intensity and diffuseness were observed in two, one and two pancreatic cases, respectively.

Among the total of 116 cases with AC, the score of staining intensity was score 0 in 47 (40.5%), score one in three (2.6%), score two in 25 (21.6%), score three in 26 (22.4%) and score four in 15 (12.9%).

Among the 116 cases with AC, the score of diffuseness of the staining was (-) in 47 (40.5%), (+) in 30 (25.9%), (++) in 21 (18.1%), (+++) in 10 (8.6%) and (++++) in 8 (6.9%) cases.

Staining was observed in 72 out of 116 cases with AC, while no staining was seen in 44 cases. No staining was seen in the gallbladder with AC.

The types of staining were cytoplasmic, membranous and secretory in 49, six and 17 cases, respectively.

No staining was seen in any of the cases with gallbladder AC (100%), in 12 cases with colon AC (92.3%), in 14 cases with stomach AC (93.3%), in five cases with MEC (50%) and in six cases with ACC (66.7%).

Staining intensity was (++) in seven cases with PRAC (77.8%) (Figure 1) and (+++) in two cases with PRAC (22.2%); (+)/(-) in one case (10%) and (+) in eight cases (80%) of EAC; (+) in three (30%), (++) in three (30%), and (+++) (Figure 2) in four (40%) of IDC; (+)/(-) in two (20%), (+) in three (30%), (++) in four (40%) (Figure 3) and (+++) in one of LAC; (+) in two (20%), (++) in three (30%), (+++) (Figure 4) in five (50%) of OSAC; (++) in three of OMAC (75%); (++) in one of CAC; (++) in one of SAC (6.7%); (+) in three and (++) in two cases of MEC; and (+) in two (22.2%) and (+++) in one (11.1%) of the adenoid cystic carcinomas. A statistically significant association was found between the organs and the staining intensity of the carcinomas stained with SHBG ($p<0,05$).

Diffuseness of the staining was (+) in two (22.2%), (++) in six (66.7%) and (+++) in one (11.1%) of the PRAC cases; (+) in nine of the EACs (90%); (+) in six (54.5%), (+++) in two (18.2%) of the PAC's; (++) in six (60%) and (++++) in four (40%) of the IDCs; (+) and (++) in one (25%), each and (+++) in two of the GBAC's; (+) in five (50%), (++) in one (10%), (+++) in three (30%) and (++++) in one (10%) of the LACs; (+) in five (50%) of the MECs; (++) in four (40%) and (+++) and (++++) in three (30%) each, in the OSAC; (+) in one (25%) and (++) in two (50%) of the OMACs. A statistically

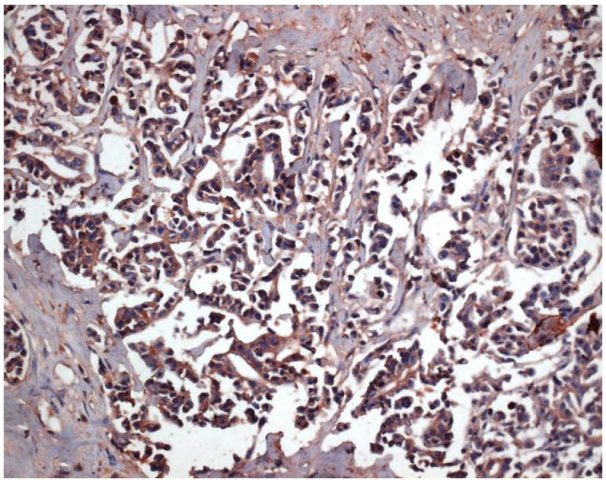

Figure 2. (++) Cytoplasmic Staining with SHBG in IDC (Immunoperoxidase x 200).

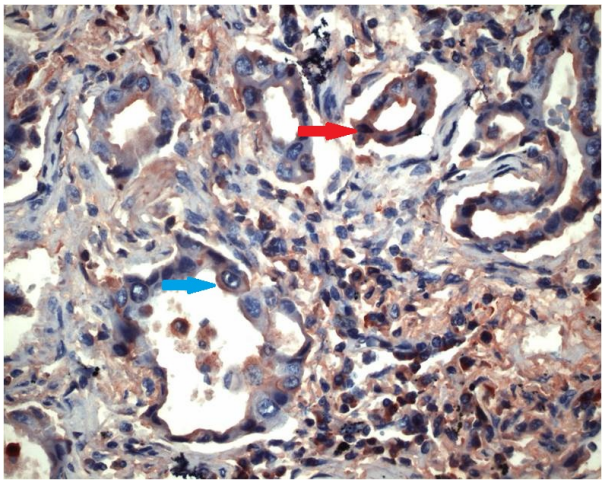

Figure 3. (++) Cytoplasmic Staining (red arrow) with SHBG and Membranous Staining (Blue arrow) with SHBG in lung AC (Immunoperoxidase x 400)

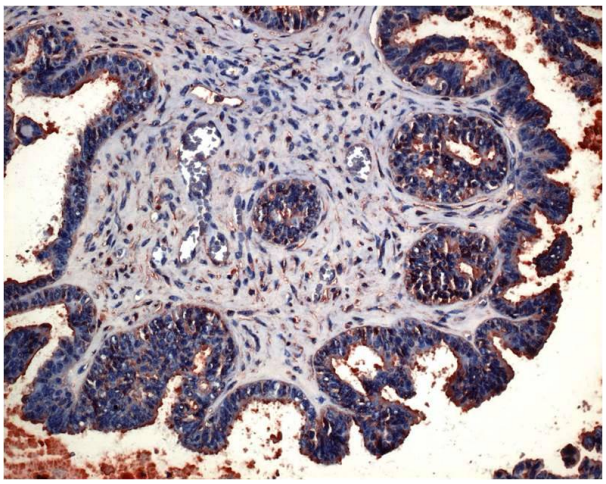

Figure 4. (+++) Secretory Staining with SHBG in Ovarian SAC (Immunoperoxidase x 200)

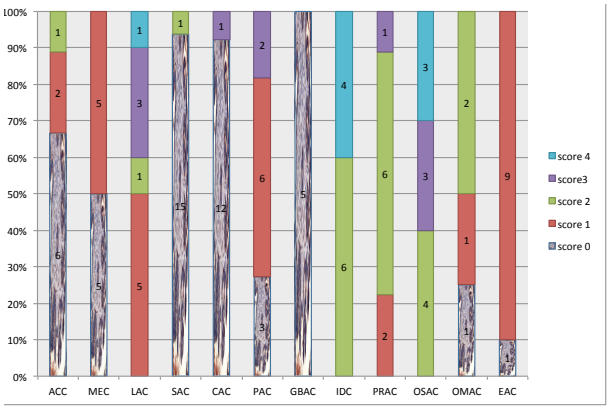

Figure 5. Distribution of Tumors by SHBG Staining Diffuseness

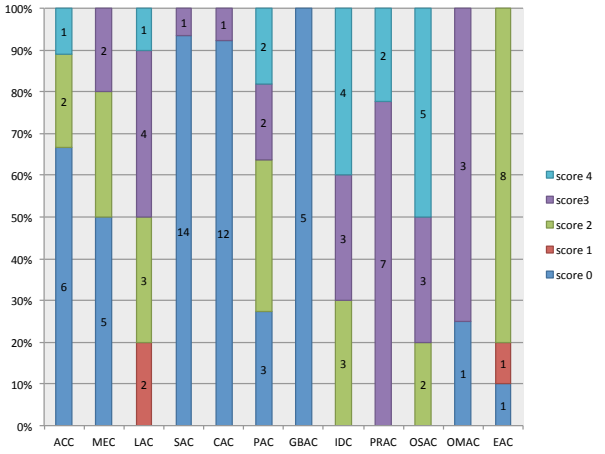

Figure 6. Distribution of Tumors by SHBG Staining Intensity

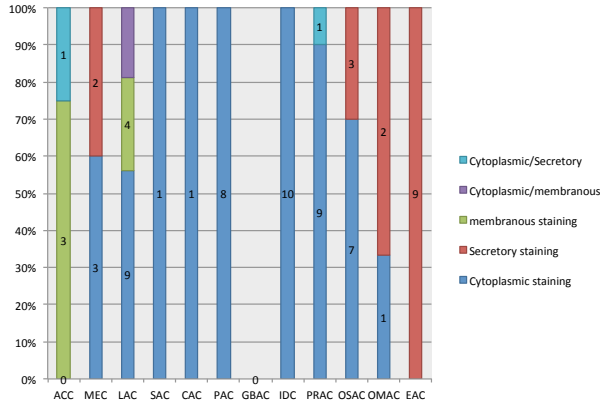

Figure 7. Distribution of Tumors by type of SHBG Staining

significant association was found between the organs and the staining diffuseness of the carcinomas stained with SHBG ($p<0,05$).

According to the staining diffuseness, stomach, colon and gallbladder ACs were observed to be in the no staining region. Pancreas, endometrium and salivary gland ACs took their places in the spatial two-dimensional graph between (0) and (+); LAC's between (+) and (+++); prostate and ovary AC's between (++) and (+++) and IDC between (++) and (++++).

According to the staining intensity, stomach, colon and gallbladder ACs were observed to be in the no staining region. Pancreas and salivary gland ACs took their places in the spatial two-dimensional graph between (0) and (++); EAC between (+) and (++), LACs between (++) and (++++); ovarian and prostatic ACs and IDC between (+++) and (++++).

Discussion

SHBG is the major plasma protein providing the transfer of biologically active sex steroids and plasma concentration of SHBG affects the amount of free sex steroids in the circulation, and half-life and the transfer of them to the target tissues (Westphal,1986; Hammond, 1995). Classical knowledge suggests that SHBG, with a half-life of 15 days, is synthesized in the liver (Pasquali et al., 1997).

Numerous studies have been conducted on the plasma levels of SHBG, the associations of these levels and various physiological functions and also some tumors (Sulkes et al. 1984; Teruel et al., 1989; Dechaud et al., 1995; Misao et al., 1995; Cousin et al., 1998).

On the other hand, IHC studies demonstrating SHBG in tissues are quite limited. They include normal and tumoral tissues of breast, prostate and ovary, demonstrating the presence of SHBG in normal breast ductus epithelial cells, prostatic gland epithelial cells, ovarian granulosa-lutein cells and invasive and noninvasive carcinomas of the breast (Sinnecker et al., 1990; Meyer et al., 1994; Hrby et al., 2002; Forges et al., 2004).

In various studies the association of plasma levels of SHBG and the risk and prevalence of some organ cancers, and the prognostic importance of SHBG levels, has been analyzed with equivocal results.

Grasso et al found significantly high serum SHBG levels in prostate carcinomas, compared with benign prostate hyperplasia, and control groups (Grasso et al., 1990). Lee et al found a significant association between serum SHBG level and the Gleason score, which histopathologically grades prostatic ACs. In the same study, SHBG levels were significantly higher in tumors extending outside the prostate, compared with tumors presenting with prostatic involvement alone (Lee et al., 2008).

In studies including an analysis of plasma levels of SHBG, which were performed with the aim of ascertaining whether high androgen level increases the risk of prostate cancer, Barrett-Connor et al. and Guess et al. found no association between testosterone, SHBG or 5α-reductase and prostate cancer risk (Barrett-Connor et al., 1990; Guess et al., 1997).

In two separate studies, the risk of prostate cancer was reported to slightly reduce with an increase in serum SHBG (Gann et al., 1996; Dorgan et al., 1998).

Literature findings have demonstrated that endometrial cancer in postmenopausal patients has a direct association with estrogen levels and an inverse relationship with SHBG levels. In cases with endometrial cancer, estrogen levels (estradiol, estron) were found to be significantly increased compared to the control group, while SHBG levels were reported to be significantly lower when compared with the control group (Austin et al., 1991; Potischman et al., 1996; Zeleniuch-Jacquotte et al., 2001) In a study performed by Ergun et al. (1995) SHBG levels were found to be significantly lower in the high risk group and in patients with endometrial cancer compared with the control group; the investigators suggested that in addition to an endometrial biopsy this might be used in the evaluation of high risk patients and also in the follow-up of patients (Ergun et al., 1995).

Misao et al, studied plasma SHBG mRNA levels in patients with normal endometrium and endometrial cancer. They found a progressively decreased SHBG mRNA levels in late proliferative phase endometrium, early proliferative phase endometrium and well, intermediate, and poorly differentiated ACs, respectively. They attributed those findings, different from other studies, to the powerful SHBG synthesis of endometrial cancer cells. Decreased

SHBG mRNA levels with lesser differentiation were interpreted as a decreased synthesis in undifferentiated cells (Misao et al., 1995).

Mahlck et al studied plasma testosterone, free testosterone, SHBG and albumin levels in patients with ovarian cancer and found increased plasma SHBG levels in those patients (Mahlck et al., 1999).

Adenocarcinomas are the carcinomas most frequently encountered by pathologists. While it might be quite challenging to find the primary origin of the tumor when these carcinomas metastasize, it might also be extremely difficult to differentiate whether the tumor is primary or metastatic in organs in which the primary cancers may frequently arise and metastasis (especially liver and lungs).

As stated above, there is no study planned to demonstrate SHBG in tissues using IHC methods, other than several studies including the normal and tumoral tissues of breast, prostate and ovary. The current study is the first to be performed using IHC methods, which included normal tissues of ten different organs including the three refered to above, and 13 different types of AC. This research aimed to establish whether the presence of SHBG in the ACs of different organs, and the demonstration of different staining patterns, affect the differential diagnosis.

Among the studies using IHC methods, which aim to demonstrate SHBG in tissues, a study performed by Meyer et al included 31 normal breasts, 21 non-invasive and 52 invasive breast cancer tissues and 33 recurrent and metastatic breast cancers. In that study, half of the non-invasive carcinoma tissues demonstrated a positive staining. Most cells were positively stained in 19.3% of the invasive carcinomas and 32.5% had no staining (Meyer et al., 1994).

In an immunohistochemical study performed by Sinnecker et al., using sex hormone-binding globulin-like antigens (SHBG-LA) in normal and neoplastic breast tissues, eleven out of 15 cases of invasive breast carcinoma demonstrated a positive staining (Sinnecker et al., 1990).

In this present study, on the other hand, all ten of the invasive breast carcinoma tissues demonstrated a positive staining. Diffuse staining (+++) and intermediately diffuse staining (++) was present in 40% and 60% of the cases, respectively. Almost half of the cases demonstrated a mild (+) and other half an intermediately intense (++) and cytoplasmic type staining.

Hryb et al. immunohistochemically demonstrated SHBG positivity in the normal and neoplastic prostate tissues and cells they cultured from those tissues. In that study, in which they also supported their findings with other methods, they interpreted the positive staining in those cells as a localized synthesis of SHBG (Hrby et al., 2002).

In our present study, all of the nine prostate tissues demonstrated cytoplasmic staining, mostly with intermediate (++) intensity and diffuseness, and one tissue demonstrated cytoplasmic staining with secretory staining with intermediate (++) intensity.

The selected organ cancers and normal and neoplastic tissues, which were IHC stained in the previous studies based on the measurement of plasma levels of SHBG, were of female and male genital system organs, which were closely related with the target organs of those hormones, due to the properties of the previously mentioned proteins of the binding sex steroids.

In the present study an attempt was made using IHC methods, to establish the presence of SHBG in organs using normal histology, including gland structures, and in ACs originating from these glands of those organs.

Among the normal tissues, no staining was noted in the salivary gland, gallbladder and colon, while staining was present in all or most of the epithelial elements of the other organs. Positive staining was seen in the luminal surfaces of the epithelial cells in the lung, prostate and endometrium, while cytoplasmic staining was present in the stomach, breast and pancreas tissues. The positively stained cells were the granulosa-luteine cells in the floor of the follicles in the ovary tissues, and three of the six stained ovary tissues demonstrated cytoplasmic and one demonstrated secretory staining. One ovarian tissue had no follicular structures and had no staining in the stroma. Among the stomach samples, on the other hand, only the parietal cells demonstrated a diffuse and strong staining, while the other cells had no staining.

When normal tissues and the ACs of those organs were compared, positive staining was present in all the ACs originating from lung and prostate and furthermore, all the normal tissues of the lung and prostate had positive staining. However, while cytoplasmic and membranous staining was present in the lung ACs, secretory type staining was present in the epithelial cells of the bronchi and bronchioles of the normal lung tissues. We suggest that this condition might be due to the difference in the tumor cells or the originating cells, or directly due to the new antigenic properties or SHBG expression ability that the tumor cells had gained.

The majority of the endometrium and pancreatic ACs were positively stained, as well as most normal tissue samples of the same organs; while all the breast invasive carcinomas demonstrated positive staining, five out of the six normal breast tissues had also demonstrated staining. All of the parietal cells showed strong staining in normal tissues, while positive staining was present in only one of the 15 stomach ACs. Normal tissues showed no staining in gallbladder tissues, as well as the gallbladder ACs, which demonstrated no staining. Positive staining was present in one of the 13 colon ACs, in five out of the ten cases of salivary gland MECs and in three out of the nine ACCs. Granulosa-lutein cells were positively stained in four out of six normal cases, while all of the ovarian SACs and most of the MACs were positively stained.

The correlation of positive staining of the organ adenocarcinomas, excluding salivary gland carcinomas and tissue epithelial cells, demonstrates to a large extent that the tumor cells reflect the SHBG staining properties of the cells of those organs from which they originated. The fact that diffuse and intense staining was seen in parietal cells of the stomach in only one of the 15 stomach ACs can suggests that a substantial percentage of these tumors did not originated from parietal cells. Positive staining detected in eight out of the 18 carcinomas of the salivary gland, in two distinct histopathological types, can be

explained by the properties acquired during the neoplastic development, since normal tissues of the salivary gland had no positive staining at all. A conspicuous property of MECs originating from the salivary gland is that, to a large extent, the staining was limited to the foci of epidermoid differentiation.

Another important question, which demarcates the comparison of normal and neoplastic cells, is whether the positive staining in those cells originates from the SHBG expression by these cells or SHBG "uptake" due to their inclusion of SHBG receptors. The classical knowledge is that SHBG is synthesized in the liver (Pasqualli et al., 1997). However, there are some published reports suggesting the presence of the extrahepatic local synthesis of SHBG. In a study conducted by Forges et al., they demonstrated the IHC positivity of SHBG in granulosa-lutein cells, suggesting that this positivity was due to the local synthesis of SHBG in these cells (Forges et al., 2004).

As was mentioned before, Hryb et al. (2002) cultured ductal and glandular epithelial cells of the prostate, establishing in a study they had reported previously the localized synthesis of SHBG and its effects (Hrby et al., 2002).

Misao et al., in their study of plasma SHBG and mRNA levels, attributed the findings of patients with normal and cancerous endometrium to the powerful SHBG synthesizing structure of endometrial cancer cells (Misao et al., 1995).

In addition to all of the above findings, the staining types, such as intracytoplasmic, membranous and apical (secretory) staining, which are seen in both normal and neoplastic tissues and have marked differences between each other, suggest the occurrence of both synthesis and uptake. However, to make this differentiation is beyond the scope of this study and can be a subject of future research.

As mentioned above, many studies have reported, with equivocal results, the search for associations between the plasma levels of SHBG and prognoses of male and female genital organ cancers (Barrett-Connor et al., 1990; Grasso et al., 1990; Misao et al., 1995; Gann et al., 1996; Guess et al., 1997; Dorgan et al., 1998; Mikkola et al., 1999; Lee et al., 2008). Likewise, research papers have also been published focusing on the search for additional information about extratumoral mechanisms and the causes of changes in the plasma SHBG levels.

Estrogens increase and androgens decrease the SHBG level and also decrease hormone-binding capacity, since SHBG is a protein structure, which provides the transport of sex steroids to the target organs (Nisker et al., 1980). Another important mechanism resulting in a decrease in SHBG levels is insulin resistance and hyperinsulinemia (Lindstedt et al., 1991). In addition to diabetes mellitus, some other causes, including obesity, nulliparity, late menopause, hypertension, polycystic ovary syndrome can also decrease serum SHBG levels and steroid binding capacity and increase estrogen-androgen levels (Ergun et al., 1995; Nisker et al., 1980; Aygen et al., 2003).

Multifactorial causes affecting the plasma levels of SHBG also explain the different results of various studies on the association of some genital organ cancers, plasma SHBG levels, and tumor prognosis. Prospective studies that will evaluate SHBG positivity in tumor tissue, as well as plasma SHBG levels in cancers of those organs and others, will probably result in more comprehensive results.

SHBG immunopositivity in the tumor cells, when evaluated alone or together with other IHC markers, clearly important in the differential diagnosis of ACs of some organs with glandular structures, and is the primary aim of this present study.

The association between the organs and staining intensity, in addition to the type of staining in this present study, was statistically significant in the differential diagnosis of the cases ($p<0.05$).

However, in spite of this significance, we consider that staining intensity and diffuseness is not very practically meaningful in the differential diagnoses, excluding gallbladder AC. Since not all cases with gallbladder AC demonstrated positive staining, it would be the first diagnosis to be ruled out in cases of positively staining AC with unknown primary (although larger series are needed due to the small number of cases in this present study).

The results of this research suggest that the differential diagnoses performed, according to the type of staining of the tumor cells, would be much more valuable.

When both cytoplasmic and membranous staining are present in the tumor in same tissue, the pathologist should be directed to lung AC and in cases where both cytoplasmic and secretory staining are present together, it would point to prostate AC.

In case of a cytoplasmic staining, the diagnoses that should be eliminated are: salivary gland ACC, endometrial AC and gallbladder AC.

In case where only secretory staining is present in a tumor, MEC, endometrium AC, and ovarian SAC and MAC should be included in the differential diagnosis.

Only membranous staining points to lung AC and salivary gland ACC should be included in the differential diagnosis.

If only the membranous staining is positive, salivary gland ACC, in which positive cases are stained only by membranous type, should be the first diagnosis to be considered. However, lung AC, which demonstrated membranous staining in only one out of six cases (16,7%), should also be included in the differential diagnosis.

If no staining is present in an AC, lung AC, prostate AC, gallbladder AC and IDC, which demonstrated 100% staining in this present case, should be eliminated from the differential diagnosis. If no cytoplasmic staining is present, prostate AC and IDC, which demonstrated 100% staining in this present case, should be eliminated from the differential diagnosis.

However, in order to reliably evaluate the role of SHBG in the differential diagnosis of ACs, more extensive studies are required, including a larger number of cases and also liver cholangiocarcinoma and cervix uteri ACs. These could not be included in our study for technical reasons.

Correlation of positive staining between ACs of organs, excluding salivary gland and tissue epithelial cells, demonstrates to a great extent that tumor cells reflect the SHBG staining pattern of the cells from which they originate. We consider that the difference between the

normal tissues and tumor tissues arise is either because the tumor cells originate from another cell type, or because of new antigenic properties, or because of the SHBG expression ability that they had directly acquired.

Intracytoplasmic, membranous and secretory staining types, which are observed in both normal and neoplastic tissues and which demonstrate a significant difference, suggest that both synthesis and uptake of SHBG should be considered in this process. However, an extensive series, with a higher number of patients, are needed to make this differentiation.

The multifactorial causes affecting the plasma levels of SHBG result in equivocal results concerning the associations between the various genital organ cancers and plasma levels of SHBG, and tumor prognosis. We suggest that prospective studies, in which SHBG positivity in tumor tissue and plasma SHBG levels would be investigated simultaneously in cases of these and other organs, will conclude in more reliable results about the prognostic importance of SHBG expression.

To reliably evaluate the role of SHBG in the differential diagnosis of ACs, more extensive studies with a larger number of cases, including cholangiocarcinoma and cervix uteri AC, are needed. This was not included in this present study due to technical reasons.

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