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E-Cadherin, beta-catenin and HER2 expression in prostate cancer tissues with perineural invasion and their correlation with Gleason score: A preliminary study

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Prostate cancer (PC) is the most common malignant tumor in men. Early identification of prostate cancer may result in improved cure rates and increased life expectancies. Gleason score and clinical range at the time of diagnosis are important factors to predict prognosis and outcome after therapy but additional accurate and reliable biomarkers are warranted. Few biomarkers of prostate cancer have been successfully implemented and used in clinical practice. In this study, we sought to determine the expression of E-cadherin, beta-catenin and human epidermal receptor (HER2) in biopsy specimens of prostate cancer with perineural invasion, and correlate them with Gleason score in order to verify the relationship between those markers and prostate cancer process. Our study demonstrated abnormal expression of E-cadherin, beta-catenin and HER2. On the other hand, our results showed no correlation between Gleason score and the expression of those markers in invasive prostate cancer tissues. Other different biomarkers remain to be identified, that potentially could improve the evaluation of prognostic of the patient.

Key words: Biomarkers, biopsy specimens, Gleason score, perineural invasion, prostate cancer.

INTRODUCTION

Prostate cancer (PC) is the most common malignant tumor in men and is a major research focus of Pathologists, Urologists and Uro-oncologists. The clinical decision of physicians, whom and how to treat these men, is dependent predominantly on pathological parameters, but still the grid spanned by these is too wide to allow a suitable prognostic of the individual case (Kristiansen, 2012). Early identification of prostate cancer may result in improved cure rates and increased life expectancies. The most widely accepted indicator for prostate prostate biopsy is a prostate-specific antigen (PSA) value > 4 ng/ml, but patients with a PSA level of ≤ 4 ng/ml, particularly 2.6 to 4 ng/ml, have clinically significant features and are well suited for immediate treatment (Nagao et al., 2011).

Gleason score and clinical range at the time of

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diagnosis are important factors to predict prognosis and outcome after therapy but additional accurate and reliable biomarkers are warranted. Despite extensive research efforts, very few biomarkers of prostate cancer have been successfully implemented and used in clinical practice. Moreover, it is unlikely that a single biomarker will provide all information needed to tell how aggressive is the newly diagnosed prostate cancer (Bjartell et al., 2011).

Epithelial (E)-cadherin is an adhesion molecule, which is expressed at the baso-lateral membrane of epithelial tissues. Intracellular interactions of E-cadherin with beta-catenin, p120, and alpha catenin also support adhesion and stabilization of the adherens junction (Grabowska et al., 2011). E-cadherin is down regulated in most epithelial cancers, and can be correlated to higher mobility and invasiveness of tumor cells (Veveris-Lowe et al., 2005). The human epidermal receptor (HER) kinase family, which includes human epidermal growth factor receptor 2 (HER2), are receptor- and receptor-like transmembrane proteins that are activated in some human tumors. The gene encoding the HER2 protein is amplified in 20 to 25% of breast cancer patients and it is overexpressed in many prostate cancers (Solit and Rosen, 2007).

In this study, we sought to determine the relative expression of E-cadherin, beta-catenin and HER2 in biopsy specimens of prostate cancer patients with perineural invasion and correlate them with Gleason score in order to find the relationship between those markers and prostate cancer process.

MATERIALS AND METHODS

Patient samples

After giving informed consent, 15 prostate cancer patients with perineural invasion were selected from 19 patients diagnosed with prostate cancer. For the purposes of this study, perineural invasion was defined as, “the presence of cancer tracking along or around a nerve” within the prostate. Perineural invasion was available on biopsy reports by two independent pathologists who were blinded to the patients' clinical information. The patient characteristics at diagnosis are listed in Table 1.

Table 1. Patient characteristics at diagnosis

Patient	Age (years)	Gleason score
1	71	8
2	42	6
3	67	7
4	64	7
5	61	9
6	61	6
7	78	7
8	72	7
9	63	6
10	60	7
11	64	6
12	72	7
13	62	7
14	66	7
15	77	7

Immunohistochemistry and histopathological analysis

Biopsy specimens from those 15 patients were immediately preserved in buffered formalin (phosphate buffer, pH 7.4) with subsequent preparation of paraffin blocks. Representative tumor areas were chosen based on hematoxylin and eosin-stained sections. The corresponding archived paraffin-embedded specimens were sectioned into 4 µm slices, and immunohistochemical staining was performed, adapted from Kowalski et al. (2003). Immunohistochemical procedures were performed using LSAB Peroxidase® with monoclonal antibodies for E-cadherin (Cell Signaling, 1:150), beta-catenin (Cell Signaling, 1:150) or HER2 (DAKO, 1:350). As negative controls, we replaced primary antibody by non-immune immunoglobin, phosphate-buffered saline or irrelevant antibodies.

More than to identify the presence or absence of a biomarker, immunohistochemical can be used to quantify its expression. Digital images can be translated into numerical values, and these values are able to describe staining intensity as a numeric variable. Considering numerical data for staining intensity and percentage of labeled cells, a combined digital immunostaining index can be defined (Matos et al., 2006). In our study, the quantification of immunostaining was made by 2 different methods: semiquantitative and computer-assisted digital image analysis. The number of stained cells per 1000 was determined under a microscope in three visual fields, at a magnification of ×200. When the total number of cancer cells observed under microscope was less than 1000, all cells were counted. E-cadherin or beta-catenin expression was interpreted as normal or aberrant (reduced or absent). Aberrant staining was defined as negative staining < 50% of the population of cells examined. Normal staining was defined as ≥ 50% staining of the cancer cells. When over 50% of all cancer cell cytoplasm was stained, the cells were considered HER2 aberrant. This cut-off value (50%) was analyzed by Software ImageLab®.

The histological grade according to Gleason score was assessed on stained sections in accordance with WHO International classification. Histologically, all tumors were adenocarcinomas. The present study was performed retrospectively, but all specimens were evaluated by two independent pathologists who were blind to the patients´ clinics characteristics.

Statistical analysis

Differences were analyzed by the Fisher´s exact test. The value of significance was taken as $P < 0.05$.

RESULTS

Patients

Table 1 shows patient characteristics at diagnosis. Mean age was 65.5 ± 6.9. Gleason score was 6 (18.18%), 7 (48.48%), 8 (24.24%) and 9 (9.09%). Gleason score = 6 was considered low grade and ≥ 7 was considered as high grade.

Table 2. Correlation between E-cadherin, beta-catenin and HER2 and Gleason score. Differences were analyzed by the Fisher´s exact test.

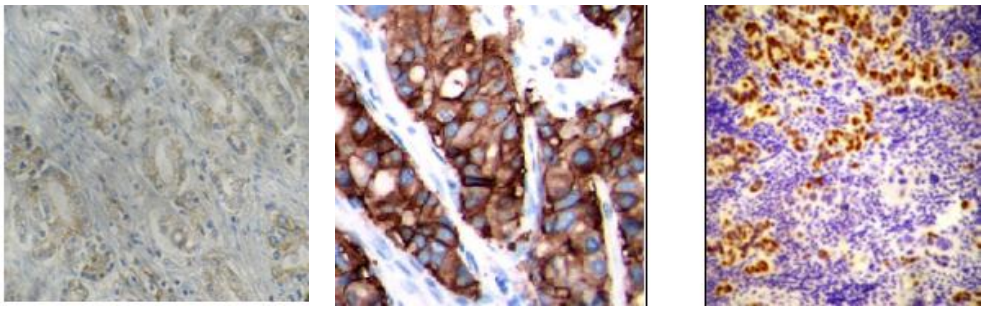
Parameter	Gleason score ≤ 6		Gleason score ≥ 7		*P*
	Normal	Aberrant	Normal	Aberrant	
E-cadherin	3	0	7	5	0.505
Beta-catenin	2	1	7	5	1.000
HER2	3	6	5	1	0.250

The value of significance was taken as $P < 0.05$. Number of patients: 15.

Table 3. Correlations between markers and Gleason score. Differences were analyzed by the Fisher´s exact test.

Parameter	Gleason score ≤ 6	Gleason score ≥ 7
	P	
E-cadherin vs HER2	0.10	1.00
E-cadherin vs beta-catenin	1.00	1.00
HER2 vs beta-catenin	0.40	1.00

The value of significance was taken as $P < 0.05$. Number of patients: 15.



(a) (b) (c)

Figure 1. Immunohistochemical expression of (a) E-cadherin (×200); (b) Beta-catenin (×200); and (c) HER2 (×200). Immunohistochemical procedures were performed using LSAB Peroxidase® with monoclonal antibodies for E-cadherin, beta-catenin or HER2. Images were analyzed by Software ImageLab®.

Immunohistochemistry and histopathological results

Relative expression of E-cadherin, beta-catenin and HER2 are shown in Figure 1. This figure is representative of the staining pattern observed for all tissue sections. When E-cadherin, beta-catenin or HER2 were considered with Gleason score (Tables 2 and 3) no correlation was observed.

DISCUSSION

Correlation between immunohistochemistry and histopathological data

There is a need f or biomarkers in prostate cancer for several reasons: (a) to improve cancer detection and staging; (b) to identify subclasses of prostate cancer; (c) to predict outcome after treatment; and (d) to select patients to different treatment opinions (Bjartell et al., 2011). The introduction of the well established biomarker PSA testing has impacted the detection rate of prostate cancer and is responsible for down-staging at diagnosis, with the vast majority of newly diagnosed tumor being localized in prostate. Moreover, Gleason score and clinical stage are important to predict prognosis and outcome after therapy but additional accurate and reliable biomarkers are necessary (Bjartell et al., 2011; Masieri et al., 2012).

The search for diagnostic or prognostic tissue biomarkers in prostate cancer was predominantly based on immunohistochemistry and a large number of tumor

markers with prognostic information were proposed. However, a vast majority of these are not used in clinical practice, probably due to lack of standardized methods to perform and interpret immunohistochemistry. In fact, the only prostate cancer biomarker routinely used in prediction models is PSA in blood (Bjartell et al., 2011). In this work, we focused on the expression of three tissue biomarkers in prostate cancer: E-cadherin, beta-catenin and HER2. Our results demonstrated abnormal expression of those markers. These discrepant results may be explained by other mechanisms, which, in turn may be important steps in the progression of cancer (Delgado et al., 2013).

E-cadherin plays critical roles in epithelial cell maintenance, and its loss from the cell surface during prostate cancer progression has been well documented (Grabowska et al., 2011). Mechanism by which a tumor cell invades the surrounding structure is poorly understood. Among numerous factors, cadherins and catenins are thought to be key molecules involved in the maintenance of the integrity of the epithelium and are likely to be involved in the earlier steps of the metastatic process. Indeed, a disruption in cadherin/catenin expression could lead to both haematogenous and/or lymphatic spread of cancer cells (Loric et al., 2001).

Mutational inactivation of alpha-catenin can be the cause of the impaired E-cadherin function, loss of catenin expression could be one of the mechanisms responsible for the loss of E-cadherin mediated cell-cell adhesion in human prostate cancer and might in some cases provide prognostic information. According to Buhmeida et al. (2006), the study should evaluate E-cadherin as a potencial biomarker of disease progression. Furthermore, there is evidence that the loss of E-cadherin adhesion results in a spontaneous increase in PSA secretion to the environment. The effects of PSA secretion may alter growth regulation and behavior of the prostate cells (Kril et al., 2001).

Our group (Serpa Neto et al., 2010) investigated the prognostic impact of HER2 over expression in patients with prostate cancer and its correlation with other pathological and clinical variables. We found a consistent association of HER2 over expression with death and recurrence. Histological grading is a very important factor for the assessment of PC prognosis. Although the reproducibility is not perfect, still the Gleason grading system is the most used prognostic factor, and highly significantly associated to survival and/or progression. When E-cadherin, beta-catenin or HER2 were considered with Gleason score (Tables 2 and 3) no correlation was observed.

Slater et al. (2003) had demonstrated that E-cadherin was unsuitable to be marker of early neoplastic transformation. In addition, our findings indicated that E-cadherin had no correlation with Gleason score. Our results differ from those observed by Nagao et al. (2011). They found a significant correlation between the low positive rate for E-cadherin and a high Gleason score. Further studies should be conducted in order to determine the real role of E-cadherin in prostate adenocarcinoma. Although markers of neoplasia are needed to improve the accuracy of diagnosis of prostate cancer, this present study adds to previous work that expression of E-cadherin, beta-catenin and HER2 could not be related to Gleason score.

Conclusion

Our results showed no correlation between Gleason score and the expression level of E-cadherin, beta-catenin and HER2 in prostate cancer tissues with perineural invasion. Other different biomarkers remain to be identified that potentially could improve the evaluation of prognostic of the patient.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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