EVALUATION OF CD44V6 AND HYALURONAN IN PATIENTS WITH BREAST CANCER: CORRELATION WITH OTHER PROGNOSTIC FACTORS.

Thesis

Submitted to the Medical Research Institute University of Alexandria For Partial fulfillment of

Doctor Degree In Applied Medical Chemistry

By

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2009

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تعيين CD44v6 والهيالورونان في مرضى سرطان الثدى وعلاقتهما بالعوامل التنبئية الأخرى.

رسالة

مقدمة إلي معهد البحوث الطبية
جامعة الإسكندرية

إيفاءاً جزئياً للحصول علي

درجة الدكتوراه
في

الكيمياء الطبية التطبيقية

مقدمة من

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بسم الله الرحمن الرحيم

Evaluation of CD44v6 and hyaluronan in patients with breast cancer: correlation with other prognostic factors.

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Introduction

Breast cancer is the most common and dreaded malignancy in women. It has an unpredictable course and characterized by long duration and heterogenicity among patients [1]. Although the breast is anatomically accessible, it metastasize very early in their course, 12-37% of small mammographically detected breast cancer are already have metastasized at diagnosis [2-3]. Moreover, metastatic disease occurs in about half of the cases with apparently localized breast cancer (stage M0) within 5 years after surgery [4]. Even among patients with node negative disease, approximately one third will recur with distant disease, as the recurrence rate in these patients has been reported to be 25%- 30% [5,6,7].

It is well known that tumor cells shedding is considered an early event in the multiphase process of metastases .The primary tumor in breast cancer sheds cells into the blood soon after it becomes invasive and these tumor cells can reach every anatomic district organ and tissue through peripheral blood circulation [8,9]. Therefore, the possibility of detecting tumor cells in the blood stream and tissue or bone marrow before clinical evidence of distant metastases is an active area of research [10].

Prediction of breast cancer outcome may be facilitated by the integration of traditional prognostic factors together with additional markers and macromolecules involved in the biologic process and tumor genesis such as, proliferation and invasion markers, epidermal growth factors, adhesion molecules and others [11,12,13].

A marker in peripheral blood of breast cancer patients that seems to have a role in prognosis is CD44.The CD44 gene, located on human chromosome 11 band p 13, is an adhesion molecule that belongs to a family of cell surface transmembrane glycoproteins members and its various isoforms (v1-v10) differ in the extracellular part [14,15].

Thus, CD44 embraces a whole family of cell surface glycoproteins, which are expressed in a wide variety of tissues and cell types. The CD44 family has been implicated in several cellular functions including cell-cell and cell matrix adhesion, migration, and tumor metastasis as it is involved in the motility and invasion of tumor cells [16-17]. It participates in fundamental biological processes including cell traffic, lymphocyte homing, haematopoiesis, inflammation and apoptosis, also, implicated in tumor pathology, playing a role in tumor cell differentiation, invasion and metastases [18,19].

Multiple functions are attributed to multiple isoforms of CD44. Splice variants containing the v6 axons were found to confer metastatic potential in experimental rat tumor system [15,20]. In human, up regulation of CD44v6 was observed in non-Hodgkin's lymphoma and colonic adenocarcinoma [21,22]. Recent studies on breast cancer have indicated a correlation between deranged CD44 expression and indicators of poor prognosis, such as tumor grade, lymph node involvement and estrogen receptor status [23]. Friedrichs et al [24], and Bankfalvi et al [25] found that, the over expression of CD 44v6 isoform significantly correlated with disease free survival. Meanwhile, they were increased in patients with metastases as compared to non-metastatic cases, and

preferentially found in patients with metastases in liver or bone [26,27]. Cells expressing a high level of CD44 isoforms also display enhanced hyaluronan binding that increases their migration capability [28].

Recently, a number of studies indicate that interaction of certain extracellular matrix components as hyaluronan with cells triggers the cytoplasmic domain of CD44 isoforms to bind unique downstream oncogenic signaling molecules and to coordinate intracellular signaling pathways leading to the onset of multiple cellular functions as tumor cell growth, migration, and invasion and breast tumor progression [29].

Hyaluronan (HA), a nonsulfated glycosaminoglycan is a component of the extra cellular matrix and is present in various tissues and tissue fluids. HA offers tumor cells some protection against immune surveillance and chemotherapeutic agents. Meanwhile, it plays a key role in motility of normal and malignant hematopoitic cells [29]. A hyaluronan rich environment often correlates with tumor progression and its interaction with its CD44 receptor and the resulting signal transduction events may be among the mechanisms for hyaluronan – associated cancer progression [29,30].

Small fragments of Hyaluronan are angioginic where these fragments induce endothelial cell proliferation, migration and lumen formation. Hyaluronidase, an endoglycosidase, degrades Hyaluronan into small angioginic fragments [31,32]. In establishing the association of hyaluronidase to tumor biology, Vinta B et al. [30], showed that hyaluronidase levels are elevated in prostate cancer and metastatic breast cancer and correlated with the aggressiveness of the disease.

Markers of submicroscopic spread in breast cancer could have potential therapeutic impact especially when combined with sentinel node assessment of axillary disease. The axillary lymph node status is still the most important prognostic factor in the staging and treatment of breast cancer and considered as the most powerful predictive factor for recurrence [33,34]. Moreover, Carcinomas expressing high levels of CD44 isoforms are more malignant than those carcinomas with a low level of CD44 isoform expression. The intra-tumoral concentration of CD44 isoforms and the hyaluronan are significantly higher in breast cancer tissues compared with fibroadenomas and normal epithelial tissues of the breast [28].

Thus, detection of peripheral blood micro metastases with the use of diagnostic and investigative techniques in combination with the traditional prognostic variables may allow identification of patients at high risk of systemic disease. Clearly, there is need to establish which markers may have potential in the diagnosis of minimal residual disease in breast cancer, as well the more effective and sensitive method which may enable effective treatment strategies.

AIM OF THE WORK

The aims of this study are:

- Study of the relationship between the measured parameters and different clinicopathological parameters.
- Study the outcome of patients as regards the metastatic potential in relation to the measured parameters.

PATIENTS AND METHODS

Patients:

The study will include 50 patients with pathologically proved breast carcinoma in different stages of the disease. 20 subjects having benign breast lesion will be included as control.

All patients will have staging work up including:

- Thorough history taking
- Complete clinical examination.
- FNAB or excisional biopsy from breast mass for pathological examination.
- Clinical staging and pathologic classification.(According to the AJCC staging system)
- Laboratory investigations: CBC, Liver profile and renal functions.
- Radiological examination including chest x-ray, abdominal US, breast mammography and US and isotopic bone scan.

Methods:

A) Assessment of CD44v6 receptor, hyaluronan and hyaluronidase in these patients will be studied before surgery. Patients will be subjected to Modified Radical Mastectomy (MRM), followed by adjuvant treatment either Radiotherapy or Chemotherapy or both ± antioestrogen.

B) Histopathological diagnosis of malignant breast specimen including different prognostic criteria of the mass, hormonal receptors study, as well as extent of lymphnode metastases and others.

I- Assessment of CD44:

a) Blood samples will be withdrawn from all subjects under study to assess CD44v6 by Enzyme- Linked Immuno-Sorbant- Assay (ELISA).

b) Paraffin sections from breast mass will be subjected to immunohistochemical stain with CD44 v6 [35].

II- Assessment of Hyaluronan:

The level of hyaluronan will be estimated in the collected samples using ELISA- like assay [36] .

III- Estimation of Hyaluronidase:

The level of Hyaluronidase enzyme will be monitored in the serum of the patients according to Jorge Benozzi et al method [37].

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