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Original Research Paper

Pathology

STUDY OF PROGNOSTIC SIGNIFICANCE OF CD44(CELL ADHESION MOLECULE) EXPRESSION IN GASTRIC ADENOCARCINOMA

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ABSTRACT

Background: Gastric adenocarcinoma is one of the most leading malignancy worldwide. Though multiple genetic aberrations were identified in the carcinogenesis of gastric cancer, the unique property of CD 44 which is a cell adhesion molecule allows the process of tumor progression and metastasis. The current study was done to study the association between CD44 expression with variable pathological features of gastric adenocarcinoma and to evaluate probable prognostic significance of it.

Methods: A retrospective review of gastric adenocarcinoma cases diagnosed histopathologically who have undergone total/subtotal gastrectomy over a period of one year was done. Tumors were classified histologically according to Lauren's classification and staged according to TNM. Appropriate paraffin blocks of tumor tissue were chosen for further evaluation for CD44 expression and it was correlated with various pathological features of gastric adenocarcinoma.

Result: Of 50 patients, eighty six percent of patients were CD44 positive. CD44 was found to be more commonly expressed in the intestinal subtype (P = 0.049). A significant corelation was seen between the histological grade / stage of tumor and the expression of CD44 (P = 0.000).

Conclusion: By statistical analysis CD44 expression was found to show significant correlation with histological type, grade as well as stage. Since the stage is a proven prognostic marker in gastric adenocarcinomas, CD44 expression can be used as a prognostic marker in endoscopic biopsy itself to assess the invasiveness and also the metastatic potential of these tumours and thereby helping to improve prognosis of patients by the application of better targeted therapy as there is still an unmet need for approaches to predict individual therapy responses.

KEYWORDS : Gastric Adenocarcinoma, CD44 expression, Prognosis, Immunohistochemistry

INTRODUCTION

Gastric carcinoma is one of the leading causes of cancer related deaths all over the world and most common cause of death is due to recurrence and metastasis. Though multiple risk factors are being identified in the carcinogenesis (most importantly genetic abnormalities), definitive etiology is still elusive. Tumor formation basically depends upon the interaction between the tumor cells and also between the tumor cell and the extracellular matrix which is regulated by cell adhesion molecules.

Cell adhesion molecule groups have been classified into 5 family of proteins which include cadherins, integrins, members of immunoglobulin family, selectins and CD44, some of which are studied extensively in detail previously[1,2,3]. Of this, CD44 has been under research rador recently, as it also has cancer stem cell like property.

CD44 is a transmembrane glycoprotein responsible for many physiological and biological functions including cellular adhesion[4]. It was initially detected in lymphocytes and hematopoietic cells facilitating lymphocyte recirculation/homing through property of cell adhesion to high endothelial venules[5]. It is a polymorphic gene mapped in short arm of chromosome 11, encoding numerous isoforms such as CD44s(standard), CD44R and CD44v(variant isoforms), each one of which plays significant role on its par[6,7,8,9]. Expression of variable isoforms of CD44 is highly restricted and specific and plays a key role in malignant transformation of epithelial cells[10]. The primary techniques that helps to evaluate the presence and degree of CD44 expression are immunohistochemistry, fluorescence cell sorting, and reverse transcriptase polymerase chain reactions (RT- PCR). Research on CD44 has been active at molecular levels, but then it is fragmented.

This study aims to use most feasible and reliable technique of immunohistochemistry which is available in many institutes to assess the level of CD44 expression in gastric adenocarcinoma and also to evaluate the relationship of CD44 expression with the pathological features especially histological type, grade and stage of gastric adenocarcinoma , thereby evaluate prognostic value of CD44 expression in gastric adenocarcinoma cases .

MATERIALS AND METHODS;

This is a longitudinal retrospective study carried out in a tertiary care hospital and Institutional Ethical committee clearance was obtained for the study. Gastric adenocarcinoma cases diagnosed histopathologically who have undergone total / subtotal gastrectomy from August 2011 to August 2012 were selected and clinicopathological data was collected with the help of prestructured proforma. Tumors were classified histologically according to Lauren's classification , graded by the WHO criteria and was staged according to TNM staging. Non adenocarcinoma cases and patients who underwent preoperative chemotherapy were excluded from the study. H&E sections of these cases were reviewed and analysed both for grading and staging. Representative formalin fixed paraffin embedded blocks of each case were taken. Appropriate blocks with maximum tumor tissue were chosen for evaluation of CD44 by IHC staining. IHC was done by Horse radish peroxidase polymer technique. Validated procedure for immunohistochemical staining were followed. Primary antibody - mouse anti-CD44 monoclonal antibody, clone DF1485 which identifies all isoforms of CD44v was used. Tonsillar tissue was taken as external positive control and tumor infiltrating lymphocytes within tumor tissue act as internal positive control[Fig.1]. Negative control was obtained by skipping application of primary antibody to one slide and normal gastric tissue was taken as internal negative control. Both positive and negative control was applied with each batch of staining. Immunoreactivity was considered positive when dark brown homogenous staining was seen located in either membranous or cytoplasmic or both[Fig.2].

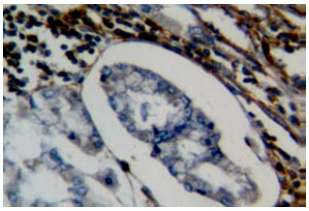

Fig.1-IHC(CD44) 40X- Negative CD44 in tumor glands but positive in infiltrating tumor lymphocytes(internal positive control)

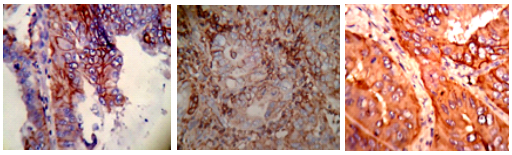

Fig.2-IHC(CD44) 40X- High/Low membranous expression, both membranous and cytoplasmic positivity

For the interpretation of CD44 expression in the tissue, both the staining intensity and percentage of staining area were considered and scored . Intensity of staining was scored as follows: (0)= No staining, (1+)= Weak staining, (2+)= Moderate staining, (3+)= Strong staining. The percentage of staining area was scored accordingly : 0= 0%; 1=when 1-10% of the area gets stained, 2=when more than 10%, but upto 50% of the tumor shows staining, 3=more than 50% but upto 100% of the tumor are being stained.

Composite score was obtained by multiplying both the intensity of staining as well as the percentage of staining. The composite score ranges from 1 to 9. Composite scores of 1-3 was labelled as 'low CD44 expression', whereas scores of 4-9 were labelled as "high CD44 expression". Tumors with both weak and moderate / strong staining is regarded as positive expression, while those with negative staining only is considered as negative expression. Statistical analysis were performed using SPSS software to correlate between the CD44 expression status and clinical variables as well as histologic parameters such as histological type, grade and stage. Results were tabulated and statistical significance were analysed for necessary variables such as CD44 expression against histological type, grade and stage. P value of less than 0.05 was considered statistically significant

RESULTS;

Out of 50 cases, 43(86%) cases showed CD44 positive expression and 7(14%) showed negativity to CD44. Out of 43 CD44 positive cases, 23(53%) cases showed low expression and rest of 20(47%) cases showed high expression. 63% of the CD44 positive cases showed membranous pattern of staining, 21% cases showing cytoplasmic positivity and rest shows both membranous and cytoplasmic staining. 75% of membranous positive cases were seen among high CD44 expression group. No significant correlation was seen between CD44 expression with respect to clinical variables of gastric carcinoma cases. CD44 expression is found to significantly correlate with intestinal type of gastric adenocarcinoma[Table 1]. The P value is 0.049 which is statistically significant. CD44 expression was seen high in almost all cases(100%) of well differentiated adenocarcinoma, whereas poorly differentiated tumors show either low(67%) or negative expression(33%)[Table 2]. The P value for this was 0.000 which is highly significant. In the 50 cases analyzed, all the stage 1 tumors showed high expression(100%) and 75% of stage 4 tumors showed negative expression[Table 3]. The P value for this was 0.000 which is statistically significant

TABLE – 1 CD44 expression vs Histological type

Histologic Type	Cd44 Expression		
	Negative	Low	High
Intestinal	6	17	20
Diffuse	1	6	0

TABLE – 2 CD44 expression vs Histological Grade

Cd44 Expression	Histological Grade(Differentiation)		
	Well	Moderate	Poor
High	7	13	0
Low	0	15	8
Negative	0	3	4

TABLE-3 CD44 Expression vs Histological Stage

Histological Stage(TNM)	CD44 Expression		
	Negative	Low	High
Stage 1	0	0	10
Stage 2	0	5	6
Stage 3	4	17	4
Stage 4	3	1	0

DISCUSSION;

CD44 (cell adhesion molecule) is a principal cell surface receptor for hyaluronic acid. Hyaluronic acid is a major component of extracellular matrix. Stroma of many of the tumors contain increased amount of hyaluronic acid. Hence increased expression of CD44 by the neoplastic cells potentiates the tumor cells to adhere to extracellular matrix through ligands like hyaluronan and allows active formation of cell colonies [11,12] .

CD44 plays a role in carcinogenesis, differentiation, as well as lymph node metastasis which are considered to be prognostic for various other carcinomas such as lung carcinoma, breast cancer,colon carcinoma, pancreatic carcinoma as well as leukemia[13,14,15]. Both the metastasizing tumor cells and recirculating lymphocytes share similar properties like motility and invasive behavior, which indicates that tumor cells might use molecules like CD44 for metastasizing[16]. Expression of variable isoforms of CD44 is highly restricted and specific and plays key role in malignant transformation of epithelial cells. CD44 also functions as a putative cancer stem cell surface marker and henceforth can be used as a novel therapeutic target[17]. To date, several studies have been reported regarding CD44 expression in various carcinomas as mentioned. This kindled interest to study the most feasible technique of immunohistochemistry to evaluate CD44 expression in gastric adenocarcinoma which is most important and burning health issue encountered frequently in our hospital tumor clinic. Treatment modalities of gastric cancer depends on clinical stage and other comorbidities and it is usually a multidisciplinary approach which will be helpful for the patient on long term basis[18]. Although chemotherapy enables tumor cell death, many patients experience recurrence which may be due to failure of these therapy to target cancer stem cells which are the key players in tumor evolution as well as responsible for recurrence. In view of better management / prognosis of the patients with gastric adenocarcinoma, development of new molecular biomarkers is thus necessary to assess the outcome of those patients so that the surgeons would manage them with intense therapeutic regimens (either medical/ surgical). There are many contradictory reports regarding the role of CD44 adhesive molecule in gastric carcinogenesis and metastasis.

All the 50 cases which were included in present study was evaluated for CD44 expression which is considered as one of the novel biomarker for assessing metastasis and prognosis in many malignancies. The present study showed 86% of CD44 positive cases of which 40% were showing high expression and all of them were intestinal type of gastric adenocarcinoma. CD44 expression showed significant statistical correlation with histological type (intestinal) [p value=0.049]. Our findings do correlate with most similar studies performed earlier by Kamran et al, Yagamuchi et al, Saito et al[19,20,21]. 54% of all CD44 positive cases were showing typical crisp membranous pattern of immunohistochemical staining which was the classic staining pattern, however CD44 also considered to be an part of cytoskeletal protein and so cytoplasmic staining was also considered positive. In our study CD44 expression was seen high in all cases of well differentiated adenocarcinoma and most of

the poorly differentiated tumors show low expression and also negative expression almost to 100%. The statistical analysis also revealed significant correlation of CD44 expression with histologic grade (well/ moderately differentiated) [p value=0.000] as well as histological stage (1,2) [p value= 0.000]. Some of the previous studies such as Kamran et al suggested that CD44 positive cases were found to have poor outcome than negative cases. However in a recent study of CD44 in many tumors established that the presence of CD44 in well differentiated carcinoma and its absence in more aggressive carcinoma suggested an inverse relation hypothesis that, if CD44 hyaluran interactions anchor cells in one area and the loss of hyaluronate binding capacity of cells lacking CD44 might relieve the cells of restraint to hyaluronate rich tissue and facilitate with metastasis[22,23]. Our study helps to prove this hypothesis and also suggests the use of this marker in endoscopic biopsies to help surgeons to further prognosticate the patient and plan for individualized therapy. CD44 expression showed no significant statistical correlation with any of the clinical variables such as age, sex, socioeconomic status and presenting illness assessed, the result of which is almost same for similar studies which were conducted in recent past.

CONCLUSION;

In gastric adenocarcinoma , loss of CD44 expression is associated with less differentiation and advanced stage of tumor and may indicate a more aggressive behavior, possibly because of loss of cell-cell or cell-matrix adhesion interactions regulated by this molecule. Hence CD44 may be used as part of prognostic panel of tests especially in endoscopic biopsies thereby alerting the operating surgeons for better vigilance. Further it helps clinician to identify subgroup of patients who may benefit from more intense post operative adjuvant therapy as well as from new advented targeted personalized therapy.

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