Article is available online at http://www.webio.hu/por/2002/8/2/0125

ARTICLE

Down Regulation of Endothelial Adhesion Molecules in Node Positive Breast Cancer: Possible Failure of Host Defence Mechanism

Maya MADHAVAN,[1] Priya SRINIVAS, Elizabeth ABRAHAM,[2] Iqbal AHMED,[3] NR VIJAYALEKSHMI,[4] Prabha BALARAM[1]

[1]Division of Cancer Research, [2]Division of Histopathology, Regional Cancer Centre, [3]Division of Surgical Oncology, Regional Cancer Centre, Trivandrum, Kerala, [4]Department of Biochemistry, University of Kerala, Trivandrum, Kerala, India

Endothelial cell adhesion molecules (CAMs) are important in tumorigenesis and host defense mechanism. Their status in breast cancer with regard to nodal invasion is not yet known. Hence we looked at the expression of three important CAMs: VCAM, ICAM and E-selectin. A downregulation of all these CAMs was noted in node positive breast cancer in comparison to node negative cases. This suggests shedding of these molecules in cases with nodal metastasis which might help the tumor cells to escape the host defense mechanism. On multivariate analysis, VCAM alone emerged as an independent predictor of nodal metastasis. (Pathology Oncology Research Vol 8, No 2, 125–128, 2002)

Keywords: VCAM, ICAM, E-selectin, invasion, breast cancer

Introduction

Breast cancer is the most common female malignancy often presenting an unpredictable course of development and progression. Identification of environmental, biochemical and genetic factors which are important in the etiology and progression of this disease is essential in improve prevention, diagnosis and therapy.

The metastatic progression of malignancy involves a cascade of sequential reactions, and interaction of malignant cells with endothelial cells or sub-endothelial basement membrane play an important role in the establishment of metastasis.[1,2,3,4] CAMs expressed on lymphocytes and vascular endothelial cells play an important role in the progression of malignancies[5,6,7] possibly by mediating tumor cell-endothelial cell interactions. The major endothelial adhesion molecules are vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1) and E-selectin. These are activated by cytokines via a NF-κB dependent pathway. These molecules are also implicated in lymphocyte trafficking for the host immune anti-infection response.[8]

V-CAM-1 is a glycoprotein with a molecular mass of approximately 110 KDa expressed on the surface of stimulated endothelial cells.[9,10] It is an adhesion molecule for many different cell types including lymphocytes,[11] monocytes, neural cells and hematopoietic cells. Its expression is induced by stimulation of endothelial cells by cytokines such as IL-1, TNF and LPS. Increased serum concentrations of VCAM have been reported in most of the cancers.

ICAM-1 is a 80-110 KDa cell surface glycoprotein strongly expressed in the endothelial cells of the small blood vessels and fibrous tissue in the vicinity of cancer cells.[12] It may exert its most important function in primary tumor tissue where it is expressed by intratumorous endothelial cells as well as by the tumor cells. On the endothelial surface, it may cause arrest of circulating lymphocytes in intratumorous sites and, on tumor cells, ICAM appears to mediate homotypic adhesion between cancer cells. The presence of ICAM on target cells is postulated to be advantageous for cell-mediated lysis in some systems.[13]

E-selectin is an endothelial membrane protein also known as endothelial leukocyte adhesion molecule-1 (ELAM-1) with a molecular weight of 97-115 KDa.[14] It is one of the three known members of the selectin family[15] which recognize carbohydrate ligands. They mediate the

Received: March 20, 2002; *accepted:* May 25, 2002

Correspondence: Dr. Prabha BALARAM, Head, Research Division, Regional Cancer Centre, Trivandrum, Kerala, India; PIN-695011; Tel: 91-0471-522203, fax: 91-0471-447454, e-mail: rcctvm@md2.vsnl.net.in.

tethering and rolling of leukocytes on vascular endothelium. Two endothelial cell adhesion molecules of the selectin family have been identified: P-selectin and E-selectin. E-selectin is expressed by endothelial cells stimulated by cytokines. It mediates the rolling of neutrophils on the surfaces of endothelial cells and initiates permanent binding to the endothelium by other adhesion receptors including ICAM.

Very little is known about the role of these endothelial adhesion molecules in the evolution of breast cancer metastasis. A number of reports are available which point out the correlations between circulating levels of CAMs and metastasis in G.I. cancer,[16] malignant melanoma[17] and Hodgkins disease.[18] But the status of these adhesion molecules in breast cancer with regard to nodal status is not yet known. Hence we studied the expression profile of these CAMs in node negative and node positive breast cancer. We show that in node-positive breast cancer, there is a significant downregulation of these adhesion molecules. Furthermore, our data suggest that VCAM-1 is an independent predictive factor of nodal metastasis.

Materials and Methods

Paraffin embedded blocks of tumor specimens from seventy primary breast cancer patients which included 33 node negative and 37 node positive cases, were randomly collected from the Department of Histopathology, Regional Cancer Centre, Trivandrum. The clinical details of the patients were collected from their medical records. Routine histology was performed and each tumor was classified according to standard norms.

5 μm sections were subjected to immunostaining using a biotin/streptavidin technique with horseradish peroxidase as the enzyme. Briefly, the sections were deparaffinised using xylene and hydrated using decreasing gradients of alcohol. The sections were washed in PBS buffer and antigen unmasking was performed by autoclave treatment for 3 minutes in 10 mM citrate buffer (pH=6). After washing, endogenous peroxidase activity was blocked by incubation with 0.3% H_2O_2 and nonspecific binding sites were blocked by incubation with 3% BSA. The sections were incubated with primary antibody overnight at 4°C, washed, and sequential incubations were carried out with a biotinylated secondary antibody and an avidin-HRP conjugated tertiary antibody. The staining was developed using AEC as chromogen and haematoxylin as counterstain. Each washing was done in 1X PBS buffer (pH=7). To ensure antibody specificity, control sections were incubated with PBS instead of the primary antibody.

Goat Monoclonal antibodies directed against E-selectin (sc-6937), ICAM(sc-1511) and VCAM (sc-1504) were used (Santa Cruz Biotechnology). The antibodies were diluted in 1X PBS in the ratio 1:30.

The staining was microscopically evaluated by two independent observers who were blinded with regard to the case status. The mean of the two staining intensities was calculated. The intensity of staining was graded as negative-1, mild positive-2, moderate positive-3 and intense positive-4 respectively.

The Mann-Whitney U test was used to determine the difference in expression of the proteins between different groups of samples. The correlations between the various proteins were determined by rank correlation. Univariate and multivariate analysis were carried out with logistic regression model to determine the potential value of the adhesion molecules as risk factors for developing node positivity. All p-values were two sided and statistical significance was declared at $p < 0.05$.

Results

The expression pattern of all the three endothelial adhesion molecules was membranous. A decreased expression of these CAMs was found in node positive cases in comparison to the node negative cases (*Table 1*). Endothelial cells of the normal blood vessels, especially large venules exhibited a fairly strong immunostaining. Looking at the bivariate correlations, ICAM showed highly significant cross-correlation with both the other endothelial adhesion molecules E-selectin and VCAM (*Table 2*).

Tumors which had a large vascular and lymphatic infiltration exhibited comparatively stronger immunostaining for all the three endothelial adhesion molecules. Among the tumors studied, there was a solitary cystosarcoma phyllodes in which the epithelial layer was strikingly negative while the stroma showed moderate positivity.

In order to determine the potential role of these CAMs as risk factors for nodal positivity, we carried out a univariate analysis using logistic regression model. Both

Table 1. **Protein expression as a function of nodal status**

Protein	*Node -ve*	*Node+ve*	*p-value*
E-selectin	2.00 ± 0.29*	1.52 ± 0.18	0.15
ICAM	2.92 ± 0.33	1.94 ± 0.27	0.03
VCAM	2.54 ± 0.33	1.58 ± 0.21	0.015

*Mean ± S.E.

Table 2. **Bivariate correlations among the adhesion molecules**

Parameters	*Correlation Coefficient*	*p-value*
ICAM / VCAM	0.6985	0.001
E-selectin / ICAM	0.5584	0.007
E-selectin / VCAM	0.5242	0.006

Table 3. **Univariate analysis of endothelial adhesion molecules as a marker of nodal metastasis**

Variable	*Regression Coefficient*	*Odds Ratio*	*p-value*
ICAM	–0.6990	0.49	0.04
VCAM	–0.8502	0.43	0.02
E-selectin	–0.5217	0.59	0.15

ICAM and VCAM seemed to be statistically significant (*Table 3*). On multivariate analysis using ICAM and VCAM, VCAM alone emerged out to be a significant independent predictor of nodal metastasis (Regression Coefficient= -0.8705; Odds ratio= 0.42; p-value=0.03)

Discussion

Reports on the expression of various endothelial molecules are rare in literature. This study demonstrates the expression pattern of these molecules in breast cancer and for the first time, a downregulation of these proteins in node positive breast cancer is reported. It is reported that ICAM is important in the early stages of endothelial formation while E-selectin is involved in the activation and differentiation of endothelial cells.[19] The significant bivariate correlation obtained between ICAM and VCAM in our study supports the fact that ICAM-1 crosslinking on endothelial cells leads to increased expression of VCAM-1 via a NF-κB independent mechanism.[20]

Circulating ICAM-1 in serum has been shown to be elevated in several diseases[21,22] and higher levels associated with metastasis, tumor spread and poor prognosis in gastrointestinal cancers,[16] melanoma,[23] uveal melanoma[24] and Hodgkins disease.[25] A significant correlation between the degree of mononuclear cell infiltration and the expression of ICAM by stromal cells in breast cancer has been reported.[26] This phenomenon is seen in our study too. Mononuclear cell infiltration in the tumor and ICAM-1 expression on tumor cells could favour an antitumor reaction by the immune system of the host. ICAM-1 was also detected in tumors without marked mononuclear infiltration suggesting that constitutive expression of ICAM-1 in breast cancer might be favourable for an anti-tumour immune response of the host as well.

Soluble E-selectin has been evaluated as a marker for endothelial damage after activation by cytokines.[27] Higher levels of E-selectin have been reported in ovarian, breast and gastrointestinal cancers.[28] E-selectin has been found to enhance ICAM-1 expression in human tumor cell lines. Wittig et al[19] reported that the enhanced adhesion of T-cells to tumor cells mediated by soluble E-selectin increased ICAM expression. Consistent with this report, we obtained a significant positive correlation between E-selectin and ICAM in breast carcinomas.

Invasive tumor cells are able to survive only if they overcome host defense mechanisms. Shedding of adhesion molecules by activated endothelial cells and tumor cells might not only block their counter ligands on immunocompetent cells, but also allow the tumor cells to escape from surveillance by cytotoxic T cells and natural killer cells thereby promoting invasion. This shedding of adhesion molecules might also prevent tumor cells from adhering to endothelial cells during extravasation.[29] We suggest that in the development of nodal metastasis, shedding of adhesion molecules occurs which aid the tumor cells escape from the host immune response and thus help the promotion of tumor dissemination and metastasis.

The results of logistic regression analysis revealed that intercellular adhesion molecule and vascular cell adhesion molecule can be used as markers of nodal positivity. However, VCAM is an independent predictor of nodal invasion. This is particularly important in view of many breast cancer patients presenting with clinically negative nodes who already have occult metastasis. Thus the use and development of these adhesion molecules as marker of nodal invasion will definitely be a boon to the clinicians. Further studies are to be carried out to elucidate the mechanism of downregulation of these CAMs in node positive breast cancer. Moreover, survival analysis of the patients with a longterm followup is to be carried out to bring out the prognostic implications of these molecules.

Acknowledgements

The financial support from STEC, Government of Kerala, India towards carrying out this work is gratefully acknowledged. Maya Madhavan is a recipient of a research fellowship from the Council of Scientific and Industrial Research, Government of India.

References

1. *Nicolson GL*: Organ colonization and cell-surface properties of malignant cells. Biochim Biophys Acta 695:113-176, 1982.
2. *Pauli BU, Augustin-Voss HG, El-Sabban ME, et al*: Organ preference of metastasis. The role of endothelial cell adhesion molecules. Cancer Metastasis Review 9:175-189, 1990.
3. *Nicolson GL*: Metastatic tumor cell interaction with endothelium, basement membrane and tissue. Curr Opin Cell Biol 1:1009-1019, 1989.
4. *Liotta LA:* Tumor invasion and metastasis of the extracellular matrix. Cancer Res 46:1-7,1986.
5. *Harning R, Mainolfi E, Brystryn JC, et al:* Serum levels of circulating intercellular adhesion molecule 1 in human malignant melanoma Cancer Res 51:5003-5005, 1991.
6. *Srivastava MD, Srivastava A, Srivastava BI:* Soluble interleukin 2 receptor, soluble CD8 and soluble intercellualr adhesion molecule 1 levels in hematologic malignancies Leuk Lymphoma 12:241-251, 1994.
7. *Koyama S, Ebihara T, Fukao K*: Expression of intercellular adhesion molecule1 (ICAM-1)during the development of invasion and/or metastasis of gastric carcinoma. J Cancer Res Clin Oncol 118:609-614, 1992.

8. *Pober JS, Cotran RS:* Immunologic interactions of T lymphocytes with vascular endothelium. Adv Immunol 50:261-302, 1991.
9. *Stoolman LM:* Adhesion molecules controlling lymphoctye migration. Cell 56:907-910, 1989.
10. *Osborn L:* Leukocyte adhesion to endothelium in inflammation. Cell 62:3-6, 1990.
11. *Osborn L , Hession C, Tizard R, et al:* Direct expression cloning of Vascular cell adhesion molecule-1, a cytokine induced endothelial protein that binds to lymphocytes. Cell 59:1203-1211,1989.
12. *Vogetseder W, Feichtinger H, Schulz TF, et al:* Expression of 7F7-antigen, a human adhesion molecule identical to Intercellular adhesion molecule-1 (ICAM-1) in human carcinomas and their stromal fibroblasts. Int J Cancer 43:768-773,1989.
13. *Makgoba MW, Sanders ME, Ginther Luce GE, et al:* Functional evidence that intercellular adhesion molecule(ICAM-1) is a ligand for LFA-1 dependent adhesion in T-cell mediated cytotoxicity. Eur J Immunol 18:637-640, 1988.
14. *Bevilacqua MP, Pober JS, Mendrick DL, et al:* Identification of an inducible endothelial-leukocyte adhesion molecule. Proc Nat Acad Sci (Wash) 84:9238-9242, 1987.
15. *Lasky LA:* Selectins: interpreters of cell specific carbohydrate information during inflammation. Science 258:964-969,1992.
16. *Tsujisaki M, Imai K, Hirata H, et al:* Detection of circulating intercellular adhesion molecule-1 antigen in malignant diseases. Clin Exp Immunol 85:3-8, 1991.
17. *Viac J, Gueniche A, Faure M, et al:* Soluble intercellular adhesion molecule 1(sICAM-1) and malignant melanoma. Cancer Letters 72:191-194, 1993.
18. *Gruss HJ, Dolken G, Brach MA, et al:* Serum levels of circulating ICAM-1 are increased in Hodgkins disease. Leukemia 7:1245-1249, 1993.
19. *Wittig BM, Treichel U, Blaheta R, et al:* Soluble E-selectin enhances ICAM-1 expression in human tumor cell lines. Exp Cell Res 237:364-70,1997.
20. *Lawson C, Ainsworth M, Yacoub M, et al:* Ligation of ICAM-1 on endothelial cells leads to expression of VCAM-1 via a nuclear factor-KB independent mechanism. J Immunol 162:2990-6.1999.
21. *Rothlein R, Mainolf EA, Czajkowski M, Marlin SD:* A form of circulating ICAM-1 in human serum. J Immunol 147:3788-3793, 1991.
22. *Seth R, Raymond FD, Mukgoba MW:* Cirulating ICAM-1 isoforms: diagnostic prospects for inflammatory and immune disorders. Lancet 338:83-84, 1991.
23. *Kageshita Y, Yoshii A, Kimura T, et al:* Analysis of expression and soluble form of intercellular adhesion molecule-1 in malignant melanoma. J Dermatol 19:836-840, 1992.
24. *Anastossiou G, Schilling H, Stang A, et al:* Expression of the cell adhesion molecules ICAM-1, VCAM-1 and NCAM in uveal melanoma: A clinicopathological study. Oncology 58:83-88, 2000.
25. *Pizzolo G, Vinante F, Nadali G, et al:* ICAM-1 tissue overexpression associated with increased serum levels of its soluble form in Hodgkins disease. Br J Haematol 84:161-162, 1993.
26. *Vogetseder W, Feichtinger H, Schulz TF, et al:* Expression of 7F7 antigen, a human adhesion molecule identical to intercellular adhesion molecule-1 (ICAM-1) in human carcinomas and their stromal fibroblasts. Int J Cancer 43:768-773, 1989.
27. *Gearing AJH, Newman W:* Circulating adhesion molecules in disease. Immunol Today 14:506-512, 1993.
28. *Banks RE, Gearing AJH, Hemingway IK, et al:* Circulating intercellular adhesion molecule-1 (ICAM-1), E-selectin and vascular cell adhesion molecule-1(VCAM-1) in human malignancies. Br J Cancer 68:122-124, 1993.
29. *Liu CM, Sheen TS, Ko JY, et al:* Circulating intercellular adhesionmolecule1(ICAM-1), E-selectin and vascular cell adhesion molecule1(VCAM-1) in head and neck cancer. Br J Cancer 79:360-362,1999.