NUHS-MD ANDERSON PATHOLOGY UPDATE

SINGAPORE

27-29 OKTOBER 2011

DR. CH'NG EWE SENG
JABATAN PATOLOGI,
PUSAT PENGAJIAN SAINS PERUBATAN

THE UNIVERSITY OF TEXAS
MD Anderson Cancer Center
Making Cancer History

NUHS
National University Health System

NUHS-MD Anderson Pathology Update

Singapore
27-29 October 2011

Organised by:

Department of Pathology, National University Health System (NUHS)

http://www.med.nus.edu.sg/path/md_anderson/welcome.html

12 CME points will be accredited

NUH NUS Yong Loo Lin School of Medicine & Faculty of Dentistry

ob. Fellowship

MD Anderson Cancer Center

Making Cancer History

NUHS
National University Health System

CERTIFICATE OF POSTER PRESENTATION

is presented to

Ch'ng Ewe Seng

Title: ***EXPRESSION OF SEMA4D IN INVASIVE BREAST DUCTAL CARCINOMA, NOS AND ITS ASSOCIATION WITH CLINICOPATHOLOGICAL PARAMETERS***

Author: ***Ch'ng Ewe Seng***

Universiti Sains Malaysia, Malaysia

NUHS-MD Anderson Pathology Update

Singapore

27 - 29 October 2011

Organized by the

Department of Pathology
National University Health System

A/Prof Teh Ming	**A/Prof Fredrik Petersson**	**Prof Cesar Moran**
BA, MD, FRCPath, FRCPA	MD, PhD	MD
Course Director	**Course Director**	**Course Director**

NUH NUS

ABSTRACT

EXPRESSION OF SEMA4D IN INVASIVE BREAST DUCTAL CARCINOMA, NOS AND ITS ASSOCIATION WITH CLINICOPATHOLOGICAL PARAMETERS

Ewe Seng Ch'ng[1], Sharifah Emilia Tuan Sharif[1], Hasnan Jaafar[1]
[1]Department of Pathology, School of Medical Sciences, Universiti Sains Malaysia, Kubang Kerian, Kelantan, Malaysia

Objectives:

In vitro and in vivo studies have shown involvement of Sema4D, a Class IV semaphorin, in tumor progression. This study aims to characterize the expression of Sema4D in human invasive breast ductal carcinoma and to evaluate its association with pertinent clinicopathological parameters.

Materials and Methods:

Expression of Sema4D in 94 patients diagnosed of invasive ductal carcinoma was evaluated immunohistochemically on paraffin-embedded sections. Three best-stained hotspots per case were selected for evaluation employing an intensity distribution score (IDS), a modified H-score system. Expression was categorized as high or low using median score as cutoff for statistical analysis.

Results:

Carcinoma cells variably expressed Sema4D. Eighty percent (228/282) of the hotspots coincided with the tumor front. The distribution of IDS of Sema4D expression was skewed (mean 230.32, standard deviation 52.31). No significant association between the levels of expression of Sema4D and age, race, tumor size, tumor grade, number of lymph node metastases, estrogen or progesterone receptor status was observed ($p > 0.05$). High expression of Sema4D was significantly associated with higher expression of the oncogene, Her-2 ($p = 0.037$).

Conclusion:

Invasive ductal carcinomas mostly express Sema4D at the tumor front. Sema4D might have prognostic significance as its expression significantly associates with Her-2 expression.

XPRESSION OF SEMA4D IN INVASIVE BREAST DUCTAL CARCINOMA, NOS ND ITS ASSOCIATION WITH CLINICOPATHOLOGICAL PARAMETERS

ve Seng Ch'ng, Sharifah Emilia Tuan Sharif, Hasnan Jaafar
partment of Pathology, School of Medical Sciences,
iversiti Sains Malaysia, Kubang Kerian, Kelantan, Malaysia

UNIVERSITI SAINS MALAYSIA

ntroduction and Objectives

na4D, also known as CD100, is a class IV semaphorin. In-vitro and in- studies have revealed that this protein plays important roles in tumor gression. It has been shown that Sema4D can initiate tumor jiogenesis, regulate tumor-associated macrophages and control invasive wth by acting through its receptor, Plexin-B1(1). Although expression of na4D in human breast cancer has been previously reported(2), it remains lear of its biologic significance in breast cancer. In this study, we aim to haracterize the expression of Sema4D in human invasive breast ductal cinoma
valuate the association between the expression of Sema4D and other tinent clinicopathological parameters.

Materials and Methods

Tumors and Patients: Ninety-four surgical resected specimens diagnosed as invasive ductal carcinoma, NOS at Hospital Universiti Sains Malaysia from the years 2006 to 2010 were included in this study. Clinicopathological data for each patient were retrieved from the formal pathology reports. This research had been approved by the institutional Research Ethics Committee (Human) with the reference number: USMKK/PPP/JEPeM[226.4.(2.10)].

Immunohistochemistry for Sema4D: Immunohistochemical staining was performed on 4um sections from each representative formalin-fixed, paraffin-embedded block. Rehydrated sections were treated with Dako REAL™ Peroxidase-Blocking Solution for 5 min to quench the endogenous peroxidase activity. Antigen retrieval was performed in a high pressure cooker, Decloaking Chamber (Biocare Medical) in DakoCytomation Target Retrieval Solution, Citrate pH 6 at 121°C for 30 seconds. Treated sections were incubated with anti-human Sema4D rabbit polyclonal antibody (Sigma, HPA015662) for 1 hr at room temperature at a dilution of 1:100 diluted with Dako REAL™ Antibody Diluent. Dako REAL™ EnVision™ Detection System (Peroxidase/DAB+, Rabbit/Mouse) was used for detection. The slides were counterstained with Meyer's hematoxylin. Human tonsil tissues were used as external positive controls.

Assessment for Sema4D expression: Using a bright-field microscope (Nikon Eclipse E600), each slide was scanned at low magnification (40x) to locate three best-stained hotspots. The locations of these hotpots were documented as whether invasive front or centre of tumor. Focusing on each hotspot using higher magnification (200x), the intensity of Sema4D staining in tumor cells was compared to that of the small lymphocytes and graded semi-quantitatively as 0 = no staining, 1= weak staining compared to small lymphocytes, and 2 = staining equal to or more than that of small lymphocytes. At least 200 tumor cells at each field were examined and the proportions of each intensity were estimated. An intensity distribution score (IDS), a modified H-score system, was calculated as $IDS = \Sigma Pi (i+1)$, where Pi is the percentage of tumor cells showing score i (i= 0, 1, 2) intensity staining(3). The IDS of three hotpots per slide were averaged to represent the final IDS score of that tumor. Cases were split into high and low expression groups using the median as cutoff for statistical analysis.

Statistical analysis: Relationships between categorical Sema4D expression and tumor variables were examined with Pearson's Chi-square. Fisher's exact test was used for lymphovascular invasion analysis due to smaller sample size. Analyses were performed using PASW® Statistics 18 software.

esults

ble 1. Characteristics of 94 cases of invasive ductal carcinoma, NOS

	N	
ge [mean(+/-SD)=52.6(+/-11.1)yrs]		
<=55 yrs	63	(67.0 %)
>55 yrs	31	(33.0 %)
ender		
Female	93	(98.9 %)
Male	1	(1.1 %)
ace		
Malay	72	(76.6 %)
Chinese	17	(18.1 %)
Indian	1	(1.1 %)
Others	4	(4.3 %)
urgical procedure		
Mastectomy with axillary clearance	79	(84.0 %)
Wide excision with axillary clearance	6	(6.4 %)
Lumpectomy with axillary clearance	1	(1.3 %)
Wide excision only	3	(3.8 %)
Lumpectomy only	5	(6.3 %)
umor size [mean(+/-SD)=65.6((+/-43.5)mm]		
<=20mm	4	(4.3 %)
>20mm but <=50mm	44	(46.8 %)
>50mm	46	(48.9 %)
ymph node metastasis [mean(+/-SD)=4.6(+/-7.0)]		
0 mets	37	(39.4 %)
1-3 mets	23	(24.5 %)
4-9 mets	18	(19.1 %)
>= 10 mets	16	(17.0 %)
ymphovascular invasion*		
Absent	12	(22.2 %)
Present	42	(77.8 %)
umor grade		
Grade I	10	(10.6 %)
Grade II	35	(37.2 %)
Grade III	49	(52.1 %)
strogen receptor		
Negative	44	(46.8 %)
Positive	50	(53.2 %)
rogestron receptor		
Negative	44	(46.8 %)
Positive	50	(53.2 %)
ler-2		
Negative	56	(59.6 %)
Equivocal	15	(16.0 %)
Positive	23	(24.5 %)

ata were available for 54 cases only

ble 2. Location of the best-stained hotspots.

	Location of hotspots	
	Invasive front	Centre of tumor
lumber of otspots	228 (80.9 %)	54 (19.1 %)

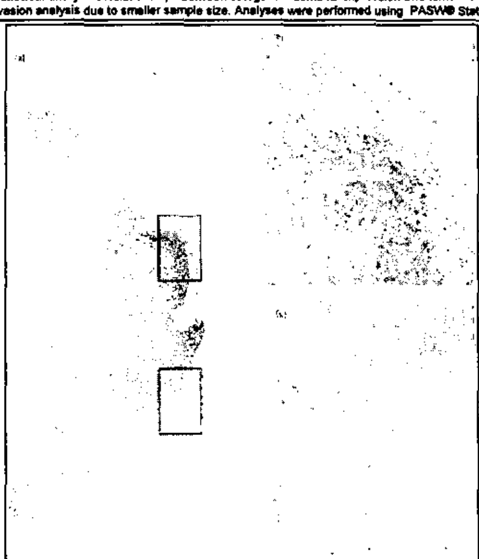

Figure 1. Invasive breast ductal carcinoma, NOS immunostained with anti-Sema4D. (a) Composite image showing variable expression by tumor cells with attenuation of staining intensity towards the centre of the tumor (40x, bar: 500um). (b)The best-stained hotspot of image (a) showing strong Sema4D expression by about 60% of tumor cells. The lymphocytes in the tumor stroma serve as internal control as well as reference (200x, bar: 100um). (c) For comparison, another area with mostly moderate Sema4D expression by tumor cells at tumor invasive front (200x, bar: 100um).

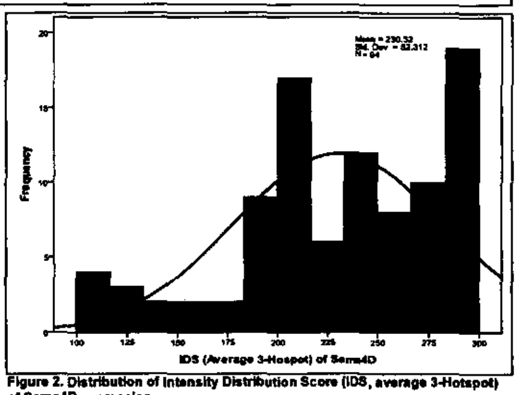

Figure 2. Distribution of Intensity Distribution Score (IDS, average 3-Hotspot) of Sema4D expression

Table 3. Association between expression of Sema4D in 94 cases of invasive breast ductal carcinoma and clinicopathological parameters

	N	Expression of Sema4D		p
		Low	High	
Age				
<=55 yrs	63	32 (50.8 %)	31 (49.2 %)	
>55 yrs	31	18 (58.1 %)	13 (41.9 %)	0.507
Race				
Malay	72	40 (55.6 %)	32 (44.4 %)	
Non-Malay	22	10 (45.5 %)	12 (54.5 %)	0.406
Tumor size				
<=50mm	48	29 (60.4 %)	19 (39.6 %)	
>50mm	46	21 (45.7 %)	25 (54.3 %)	0.152
Lymph node metastasis				
Absent	37	20 (54.1 %)	17 (45.9 %)	
Present	57	30 (52.6 %)	27 (47.4 %)	0.893
Lymph node metastasis				
0 mets	37	20 (54.1 %)	17 (45.9 %)	
1-3 mets	23	13 (56.5 %)	10 (43.5 %)	
4-9 mets	18	9 (50.0 %)	9 (50.0 %)	
>= 10 mets	16	8 (50.0 %)	8 (50.0 %)	0.969
Lymphovascular invasion				
Absent	12	6 (50.0 %)	6 (50.0 %)	
Present	42	20 (47.6 %)	22 (52.4 %)	1.000
Tumor grade				
Grade I & II	45	22 (48.9 %)	23 (51.1 %)	
Grade III	49	28 (57.1 %)	21 (42.9 %)	0.423
Estrogen receptor				
Negative	44	24 (54.5 %)	20 (45.5 %)	
Positive	50	26 (52.0 %)	24 (48.0 %)	0.805
Progestron receptor				
Negative	44	25 (56.8 %)	19 (43.2 %)	
Positive	50	25 (50.0 %)	25 (50.0 %)	0.509
Her-2				
Negative	56	33 (58.9 %)	23 (41.1 %)	
Equivocal	15	10 (66.7 %)	5 (33.3 %)	
Positive	23	7 (30.4 %)	16 (69.6 %)	0.037

*Fisher's exact test
Others: Pearson's Chi-square

iscussion & Conclusions

Invasive breast ductal carcinomas variably express Sema4D

xpression of Sema4D in human cancers was explored only in a number of studies. In one study, between 37.5% and 85.4% of human oral, prostate, colon, breast, d lung carcinomas exhibited moderate to strong Sema4D expression. However, details regarding quantification of Sema4D expression were not available(2). ifferent assessment methods to define positive Sema4D expression have been reported in prostate cancer and soft tissue sarcoma(4,5). In this study, we monstrated that invasive breast ductal carcinomas variably expressed Sema4D. Within each tumor, the tumor cells also showed variable staining intensity with toplamic and membranous staining pattern. Employing a scoring system incorporating the percentage and intensity of staining - the intensity distribution score (IDS), antification of Sema4D expression was performed. The distribution of IDS of Sema4D expression was skewed to the right. It is understandable because a selection as towards the best-stained areas will result in skewness. This distribution of Sema4D expression however did not show bimodal distribution where a cutoff could be t. Therefore, the median score was chosen as cutoff to split the cases into high and low expression groups to attain objectivity in the analysis later. In addition, in ost cases, tumor cells at the tumor invasive front expressed higher levels of Sema4D as 80.9% of best-stained hotspots coincided with the tumor invasive front. tratumoral heterogeneity, occurrence of regional phenotypic differences within the same tumor, is a known phenomenon in breast cancers. It has been shown that ognostic and predictive factors in breast cancer such as ER, PR, HER-2, p53, and MIB-1 has significant intratumoral heterogeneity(6). Immunostaining with oliferative markers such as MIB-1 and proliferating cell nuclear antigen (PCNA) has higher staining rate in the tumor invasive front as compared to the centre of the mors(7,8). In this study, however, we were unable to clarify the biological significance of selective expression of Sema4D in invasive front of most breast cancers.

Higher Sema4D expression is significantly associated with Her-2 overexpression in invasive breast ductal carcinoma, NOS

previous in-vitro study had shown that Plexin-B1, the receptor of Sema4D, could modulate the migratory ability of breast cancer cells by coupling with Her-2 in the esence of Sema4D as a soluble ligand(9). In contrast, we evaluated the expressions of Sema4D and Her-2 in tumor cells in this study. We observed that higher ema4D expression group was significantly associated with Her-2 overexpression. The biologic significance of co-expression of these molecules in tumor cells has ot been previously investigated. It can be postulated that Sema4D expressed on tumor cells may act in a paracrine manner on the adjacent tumor cells that express er-2 and Plexin-B1 to affect the biologic behavior of the tumor cells. Further studies are needed to elucidate this postulation in light of significant association between ema4D and Her-2 expression as revealed in this study.

cknowledgements

This work was supported by Universiti Sains Malaysia Incentive Grant and Short Term Grant (304/PPSP/61310048).

References

(1).Ch'ng ES, et al. [illegible] Cancer 2010;9(1):251. (2).Basile JR, et al. Semaphorin 4D provides a link between axon guidance processes and tumor-induced angiogenesis. Proc. Natl. Acad. Sci. U. S. A. 2006;103(24):9017-9022. (3).Leek RD, et al. Macrophage infiltration is associated with VEGF and EGFR expression in breast cancer. J. Pathol. 2000;190(4):430-6. (4).Wong OG, et al. Plexin-B1 mutations in prostate cancer. Proc. Natl. Acad. Sci. U. S. A. 2007;104(48):19040-5. (5).Ch'ng E, et al. Prognostic significance of CD100 expression in soft tissue sarcoma. Cancer 2007;110(1):164-72. (6).[illegible] A, et al. Intratumoral heterogeneity of immunohistochemical marker expression in breast carcinoma: a tissue microarray-based study. Appl. Immunohistochem. Mol. Morphol. 2010;18(5):433-441. (7).[illegible] S, et al. Proliferating-cell nuclear antigen (PC10) immunostaining and other proliferation indices as prognostic factors in breast cancer. J. Cancer Res. Clin. Oncol. [illegible]. (8).Connor AJM, et al. Intratumoural heterogeneity of proliferation in invasive breast carcinoma evaluated with MIB1 antibody. The Breast [illegible]:171-176. (9).Swiercz JM, et al. ErbB-2 and Met Reciprocally Regulate Cellular Signaling via Plexin-B1. J. Biol. Chem. [illegible]