Reduced expression of semaphorin 4D and plexin-B in breast cancer is associated with poorer prognosis and the potential linkage with oestrogen receptor

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Abstract. Involvement of semaphorin 4D (Sema4D) and the receptor proteins of the plexins B family (plexin-B1, -B2 and -B3) in solid tumours suggests they play a role in breast cancer. In the present study, the expression of Sema4D and plexin-Bs was examined in a breast cancer cohort. The expression of Sema4D and plexin-Bs was examined in 147 tumours together with 22 normal mammary tissues using quantitative PCR along with clinicopathological patient data, as well as in MCF-7 and MDA-MB-231 cell lines treated with selective oestrogen receptor modulators (SERMs). The expression of Sema4D, plexin-B1 and -B2 was markedly reduced in tumours with local recurrence, compared to the patients that remained disease-free. The reduced Sema4D expression was associated with poorer disease-free survival (median, 111.6 months, 95% CI, 96.5-126.7), compared to the patients with a higher expression (median, 144.0 months; 95% CI, 130.8-157.3; p=0.033). A reduced expression of plexin-B1 was observed in tumours with poorer differentiation and was associated with poorer overall and disease-free survival. No similar association was identified in relation to plexin-B2 and -B3. A higher expression of Sema4D and plexin-B1 was observed in the ERα-positive tumours compared to the ERα-negative tumours. The expression of these molecules was largely regulated in breast cancer cells exposed to SERMs. A decreased expression of Sema4D, plexin-B1 and -B2 was associated with local recurrence and poor prognosis. Response to SERMs indicated potential perspectives of these molecules in clinical assessment and management of diseases.

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Introduction

Breast cancer is a heterogeneous disease influenced by genetic and environmental factors (1). Tumour metastasis is regulated by a set of non-randomized events, starting from loss of cancer cells adhesion at the primary site, local invasion, intravasation, survival in circulation, extravasation and colonisation at distant sites. The nervous and vascular system share several anatomical and developmental similarities as these systems are combined in neurovascular bundles and in peripheral tissues. Notably, these shared developmental links also assist scientists to speculate the involvement of certain molecules controlling growth and migration of nerves in cancer cell proliferation, differentiation and dissemination (2,3). Apart from acting as axonal guidance cues, the involvement of semaphorins and plexins in altering the cytoskeleton, organization of actin filaments and microtubule networks in non-neural systems has also been observed (4,5).

Semaphorins are classified as secreted, transmembrane or glycosylphosphatidylinositol-linked proteins containing a phylogenetically conserved extracellular 'sema' domain. These molecules are further delineated into eight classes, of which classes 3-7 are present in vertebrates (6). Plexins are transmembrane protein receptors for semaphorins grouped further under A to D categories. Previously, the two families were initially identified as axon guidance cues in the nervous system and were later found to be involved in other systems including vascular, reproductive and immune systems (4,7-9).

Aberrant expression of these molecules was also associated with different diseases. For example, mutations of plexin-A2 and Sema3D have been observed in schizophrenia and anxiety (10,11). Similarly, involvement of semaphorin 4D (Sema4D) and plexin-B1 in the perineural invasion of tumour cells and angiogenesis has been established (12). Sema4D also termed CD100, constitutes 863 amino acids containing a transmembrane, an immunoglobulin (Ig)-like and a sema domain (13). Apart from their role in axonal guidance, Sema4D and plexin-B1 interactions have also been found to be responsible for T-cell proliferation (14) and B-cell survival and aggregation (15). A higher expression of Sema4D has been observed in T cells while its lowest level is evident in mature

B cells, macrophages and dendritic cells (6). P1exin-B1, a transmembrane protein is present on plasma membrane in the majority of cell types and acts as a binding receptor for Sema4D (6,17). Plexin-B2 and -B3 are the binding receptors for Sema4C and Sema5A, respectively (18-20). Altered expression patterns of these molecules have been observed in different types of tumours, as well as various cancer cell lines. Cancer cell proliferation and tumour-related angiogenesis may vary to a greater extent under the influence of these molecules.

Increased Sema4D and plexin-B1 expression in pancreatic ductal adenocarcinoma patients is correlated with lymph node involvement and metastasis (21). Similarly, in soft tissue sarcoma patients the elevated expression of Sema4D is associated with an increased mitotic division of cancer cells. Higher Sema4D levels are correlated with poorer overall and disease-free survival (22). Mammary cancer cell proliferative, angiogenic and metastatic abilities were well compromised under Sema4D knockout. A role for Sema4D as an oncogene responsible for invasiveness, metastasis and angiogenesis progression in mammary cell lines (66cl4, 4T1 and 168FARN) was also established (23). However, a significant effect of Sema4D as a guardian against metastatic progression has also been observed in a mammary tumour cohort (24). Contrasting findings regarding the expression profile of Sema4D and the plexin-B family in relation to different types of tumours have also been reported. For example, an increased Sema4D and plexin-B1 expression has also been observed in prostate (25-27), cervical (28), and breast and ovarian cancers (29). A combined effect of increased plexin-B1 along with c-Met was associated with poor cell differentiation and higher lymph node metastasis. The co-expression of the two proteins was correlated with unfavourable outcomes according to a study of 50 breast and ovarian neoplasms using immunohistochemistry and immunofluorescence staining (29). The interactions among Sema4D, plexin-B1 and Met were also responsible for triggering tumour invasive growth and metastasis (25). However, in renal and breast cancer patients, a reduced expression of plexin-B1 was observed in relation to disease progression (30-32). Plexin-B2 also shares a structural homology with plexin-B1 and its interaction with Sema4D has also been established (33).

In the present study, the expression profile of these molecules (Sema4D, plexin-B1, -B2 and -B3) was examined in a breast cancer cohort. The aim of the present study was to provide a thorough insight into the expression profiles of these molecules and their association with breast cancer progression, disease stage, cell differentiation, nodular involvement, local recurrence and bone metastasis.

Materials and methods

Collection of breast cancer specimens. Mammary tissue samples (n=169) were collected immediately after surgery and stored at -80˚C until further use, with prior approval from the local Ethics Committee. Breast cancer tissues (n=147) and background normal breast tissues (n=22) were verified by a consultant pathologist. A routine follow-up was carried out after surgery with a median follow-up period of 120 months. A higher incidence of ductal carcinoma tissues was observed in this cohort. Grading along with Nottingham prognostic index (NPI) values were evaluated by independent histologists aided with clinical and laboratory reports. Data regarding cohort are provided in Table I.

Tissue processing and extraction of RNA and generation of cDNA. Approximately 20 sections from each tissue sample were homogenised in an RNA extraction solution using a hand held homogeniser for RNA isolation. RNA quantification was carried out using a UV spectrophotometer (WPA UV 1101; Biotech Photometer, Cambridge, UK). Reverse transcription (RT) was performed from 1 μg of total RNA using a Reverse Transcription kit (AbGene Laboratories, Essex, UK).

Conventional PCR. The quality of generated cDNA was verified using GAPDH primers (Table II). Reaction conditions started with an initial denaturation of 5 min at 94˚C followed by 35 cycles of 10 sec at 94˚C, 30 sec at 55˚C for annealing and 30 sec at 72˚C, with a final elongation of 72˚C for 10 min. Thermal cyclers used in this regard were obtained from Perkin-Elmer, Surrey, UK. Amplified products were then separated on a 2% agarose gel and visualized under ultraviolet light following ethidium bromide staining.

Quantitative PCR. This study was based on the Ampliflour™ technology which was performed using the StepOne™ system (Applied Biosystems, Foster City, CA, USA). The methodology for this study has previously been optimized to quantify the transcript copy number in the mammary carcinoma specimens, as previously reported (34). Briefly, a set of standards along with the negative controls were included in this study. Beacon Designer software (Premier Biosoft International, Palo Alto, CA, USA) was used to design the primer pairs provided in Table II. A short stretch of sequence, complementary to universal Z probe, was also incorporated at the 5'-end of each reverse primer (InterGen, Inc., Purchase, NY, USA). The reagents used for this reaction included 2X concentrated Hot-start Q-Master mix (AbGene Laboratories), 10 pmol of specific forward primer, 1 pmol of reverse primer, 10 pmol of FAM-tagged universal probe and cDNA. The qPCR conditions were 95˚C for 15 min, followed by 60 cycles at 95˚C for 20 sec, 55˚C for 30 sec and 72˚C for 20 sec. qPCR for GAPDH was also performed on the same samples to normalise for any residual differences in the initial quantification of cDNA, as previously reported (34,35).

Effect of SERMs on cancer cell lines. The effect of oestrogen receptors on the expression of Sema4D and plexin-B1 was studied using MCF-7 and MDA-MB-231 breast cancer cell lines. These cell lines were purchased from the European Collection of Animal Cell Culture (ECACC; Salisbury, UK). The lines were routinely maintained in Dulbecco's modified Eagle's medium (DMEM)/F12 medium with 10% foetal calf serum (FCS). Selective oestrogen receptor modulators (SERMs) used in the present study included an agonist and antagonist for ERα and ERβ receptors. PPT (agonist) for ERα receptor while ERβ041 (agonist) for ERβ receptor were purchased from Tocris Biosciences (Bristol, UK). The SERMs were dissolved in a DMEM at 4X concentration in reference to their respective IC_{50} value.

Table I. Expression of Sema4D, plexin-B in breast cancer cohort.

Clinicopathological status	No.	Transcripts (copies/ μl, mean ± SD)			
		Sema4D	Plexin-B1	Plexin-B2	Plexin-B3
Tissue samples					
Normal	22	29.4±22.4	3235±1906	167±123	0.701±0.292
Tumour	147	24.82±4.79	2548±976	178.2±42	1.910±0.424
Tumour grade					
1	21	18.19±6.31	12111±5204	137.8±67.5	0.316±0.137
2	43	18.82±6.73	664±530[a]	149.5±38.7	2.624±0.989[a]
3	58	31.79±8.58	602±262[a]	220.2±81.4	2.023±0.520[a]
TNM staging					
I	70	29.77±6.99	4544±1793	179.7±35.8	2.041±0.649
II	40	15.99±5.22	376±200[a]	104.2±32.4	2.018±0.746
III	7	11.44±5.84	66.9±32.2[a]	80.7±64.5	0.806±0.224
IV	4	12.8±12.2	14.44±8.56[a]	28±18.0[a]	0.807±0.694
NPI (score)					
1 (<3.4)	66	20.40±6.63	2708±1678	107.2±27.8	1.801±0.603
2 (3.4-5.4)	38	28.68±6.56	2861±1489	201.9±48.5	1.451±0.600
3 (>5.4)	16	38.0±20.3	1903±1311	439±275	3.80±1.64
Clinical outcomes					
Disease-free	90	28.11 ±6.27	3262±1358	200.1±57.6	1.737±0.438
With metastases	7	3.77±2.86[a]	714±681	62.8±48.2	7.08±4.31
With local recurrence	5	3.12±2.14[a]	162±102[a]	16.5±10.6[a]	0.951±0.761
Died of breast cancer	16	26.6±10.3	645±463	261.4±72.7	1.613±0.670
Poor prognosis	28	16.44±6.19	565±301	160.8±46.0	2.96±1.26

[a]P<0.05. Sema4D, semaphorin 4D; TNM, tumor-node-metastasis; NPI, Nottingham prognostic index.

Table II. Primer sequences used in the present study.

Gene	Sense primers (5'-3')	Antisense primers (5'-3')
Sema4D	ctcagcagggaacaagact	actgaacctgaccgtacactccagctctgcatcatc
Plexin-B1	gaggtggcctacatcgag	actgaacctgaccgtacagtggtctgagccacagg
Plexin-B2	gaagacaccatccacatc	actgaacctgaccgtacaatgcacgtcaaagatgaag
Plexin-B3	ctcaacctgggcatcag	actgaacctgaccgtacaggctcgcagtacaggtg
GAPDH	ggctgcttttaactctggta	gactgtggtcatgagtcctt
GAPDH (q-PCR)	ctgagtacgtcgtggagtc	actgaacctgaccgtacagagatgatgaccctttg

A duplicate set of 6-well plates was used for this study. The cells ($5x10^5$) from each cell line were seeded in the well separately. The plates were incubated at 37˚C in a normal DMEM/F12 medium for a minimal of 24 h. The cells were later exposed to serum starvation for170 1 h duration. These wells were then exposed to a variable concentration of SERMs. After 4-5 h of treatment, the cells were lysed in total RNA isolation reagents for RNA isolation.

Statistical analysis. Statistical analysis was carried out using the Minitab statistical software package (version 14). Non-normally distributed data were assessed using the Mann-Whitney test (IQR), whereas the Student's t-test (mean ± SD) was used for normally distributed data where appropriate. P<0.05 was defined as statistically significant. A Kaplan-Meier survival analysis was carried out using SPSS statistical software (version 12; SPSS, Inc.).

Results

Aberrant expression of Sema4D and the plexin-B family in breast cancer. Transcript levels of Sema4D and plexin-B2 in the breast tumours were similar to their expression in the normal background tissues. Plexin-B1 appeared to be

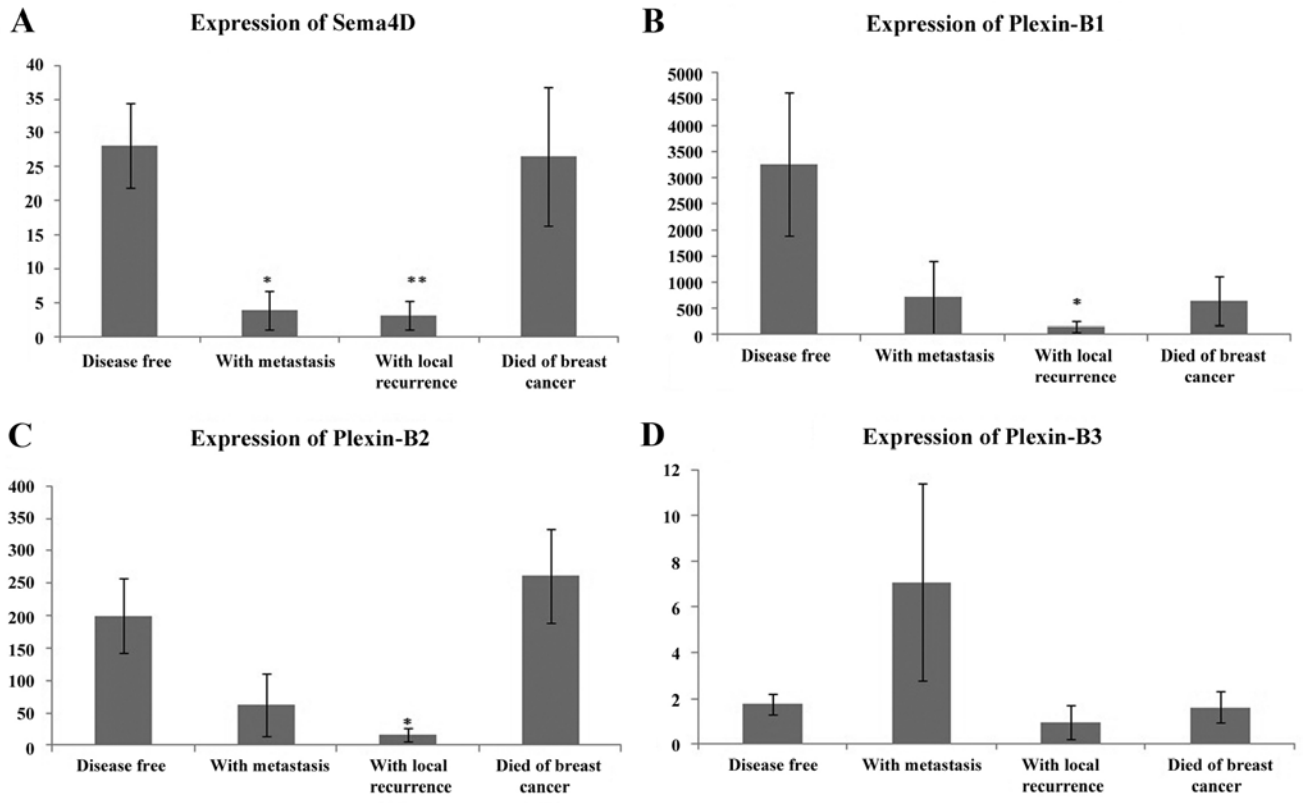

Figure 1. Relationship of Sema4D and the plexin-B family with clinical outcomes. (A) Reduced Sema4D mean transcript values in patients with metastasis (p=0.0008) and patients with local recurrence (p=0.0003) when compared with disease-free patients. (B) Significant reduction of plexin-B1 in patients with local recurrence as compared to disease-free patients (p=0.026). (C) Significant reduction of plexin-B2 in patients with local recurrence as compared to disease-free patients (p=0.002). (D) No statistically significant correlation of plexin-B3 with metastasis and local recurrence. Sema4D, semaphorin 4D.

expressed at relatively lower levels in the tumours. Notably, plexin-B3 was upregulated in the tumours, p=0.02 when compared to the control (Table I).

Correlation of Sema4D and the plexin-B family with differentiation of breast cancer cells. Transcript levels of Sema4D did not show any significant variations among well, moderately and poorly differentiated tumour tissues (Table I). The expression of plexin-B1 was markedly reduced when compared among well (grade 1) and both moderate (grade 2) and poorly differentiated (grade 3) tumour tissues, respectively (Table I). No significant correlation of plexin-B2 with tumour grading was observed. A pronounced increase of plexin-B3 expression was observed in moderately and poorly differentiated tumours in comparison to well-differentiated tumours. This increase of plexin-B3 was also statistically significant among grade 1 vs. 2 p=0.026; and grade 1 vs. 3 p=0.0024, respectively (Table I).

Expressional variations among different breast cancer types. Increased expression of Sema4D and plexin-B1 was observed in ductal (n=87) when compared with lobular (n=12), muscin (n=4), medullary (n=2), tubular (n=1) and other types of breast cancer patients (n=7). A significant correlation of the expression of Sema4D and plexin-B1 among ductal versus all previously mentioned types, excluding lobular cancer, was also established in the cohort (p<0.001). Similarly, plexin-B2 and -B3 molecules showed the highest expression in ductal cancer patients when compared with other types of breast cancers. A significant correlation of the plexin-B2 and -B3 expression levels among ductal versus all earlier mentioned types, excluding lobular cancer, was also established in the cohort (p<0.001).

Correlation of tumor-node-metastasis (TNM) staging. Transcript levels of Sema4D, plexin-B1, -B2 and -B3 tended to be reduced in the tumours at more advanced stages according to the grouping of TNM stages. Significant associations were observed in levels of plexin-B1 and -B2. The highest transcript levels of plexin-B1 were evident in tumours at an extremely early stage (TNM1), with its expression level being reduced during disease progression. The lowest expression of plexin-B1 was seen in the most advanced diseases. Similarly, the lowest expression levels of plexin-B2 were evident in the tumours with metastases (Table I).

Relationship with the clinical outcome of breast cancer. A reduced expression of Sema4D was strongly correlated with distant metastasis (p=0.0008) and local recurrence (p=0.0003) in comparison to its expression in disease-free patients (Table I). The expression of plexin-B1 downregulation was significantly associated with disease-free survival. No significant association of the plexin-B2 and -B3 expression profiles with the clinical outcome and disease-free survival were observed in the breast cohort. The reduced expression of plexin-B2 was significantly correlated with local recurrence in comparison to the disease-free one (p=0.002) (Fig. 1).

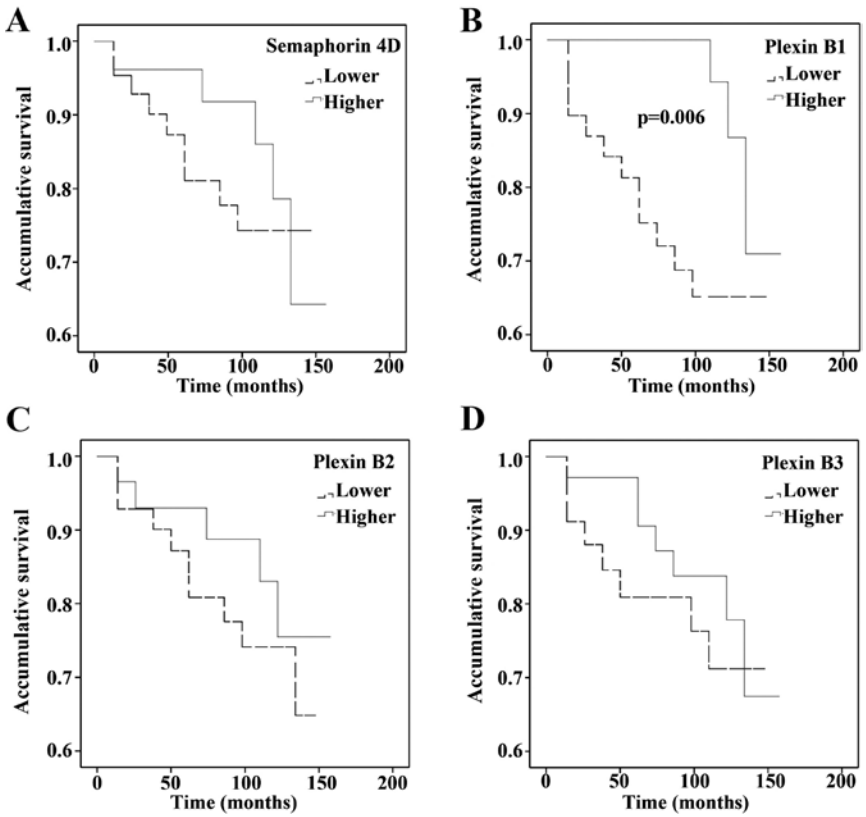

Figure 2. Overall survival curve for the cohort. (A) No significant correlation of the Sema4D expression with patients overall survival rate. (B) An increased survival rate of breast cancer patients was observed in the patients showing strong plexin-B1 expression in comparison to the plexin-B1-negative patients. The Kaplan-Meier survival curve after 10 years of follow-up. (C) No significant association of the plexin-B2 expression with the overall patient survival. (D) No significant association of the plexin-B3 expression with overall patient survival.

Expression of Sema4D and the plexin-B family with overall survival. The effect of the altered expression pattern of these molecules on patient survival was also carried out using the Kaplan-Meier survival curve (Fig. 2). Patients with an increased expression of Sema4D showed an increased disease-free survival when compared with patients stating reduced or almost negative Sema4D (p=0.033). However, the plexin-B1-reduced expression was significantly associated with worst outcome in the cohort (p=0.006). No significant relationship of plexin-B2 and -B3 with overall survival was observed.

Effect of Sema4D and the plexin-B family on bone metastasis. A significant correlation of the Sema4D expression with bone metastasis was observed in the cohort (p=0.0013, Fig. 3A). Patients with bone metastasis showed a decreased Sema4D expression when compared with disease-free patients. However, no significant association between plexin-B1 and bone metastasis was seen, although the levels were much lower in patients with bone metastasis compared to disease-free patients (p=0.069, Fig. 3B). Of note, a reduction in plexin-B2 was significantly correlated with bone metastasis (p=0.039, Fig. 3C). Plexin-B3 upregulation was observed in patients with bone metastasis, but was not found to be statistically significant (p=0.66, Fig. 3D).

Relationship with oestrogen receptor. A significant increase in the Sema4D expression in ERα-positive ductal breast cancer patients (n=57) compared with ERα-negative ductal carcinoma patients (n=23) (p=0.044) was observed in the clinical cohort. Higher levels of plexin-B1 were also observed in the ERα-positive patients as compared to the ERα-negative patients (p=0.05). Although the expression of Sema4D and plexin-B1 appeared to be lower in the ERβ-positive tumours, no statistical difference was observed in the analysis. By contrast, the expression profiles of Sema4D and plexin-B1 and the expression levels of plexin-B2 were similar in the tumours of different ER status. Among the four genes, the plexin-B3 expression appeared to be inversely linked to ERα status, and its expression was upregulated in the ERβ-positive tumours (Fig. 4).

Regulation of Sema4D and plexin-B1 by SERMs. The expression of Sema4D and plexin-B1 was determined in MCF-7 and MDA-MB-231 cells following exposure to different SERMs. Increased transcription of Sema4D was observed in the MCF-7 cells treated with PPT (ERα agonist) compared to the control (Fig. 5A). Notably, the inverse correlation of Sema4D expression with ERβ041 (ERβ agonist) treatment was observed in the MDA-MB-231 cells (Fig. 5B). This finding was also in agreement with the correlation between the Sema4D and ER receptors observed in the breast cancer cohort. Similarly, the transcription of plexin-B1 was upregulated following exposure of the PPT (agonist)-treated MCF-7 cells when compared to the controls (Fig. 5C). Plexin-B1 transcription was also upregulated by the ERβ receptor agonist

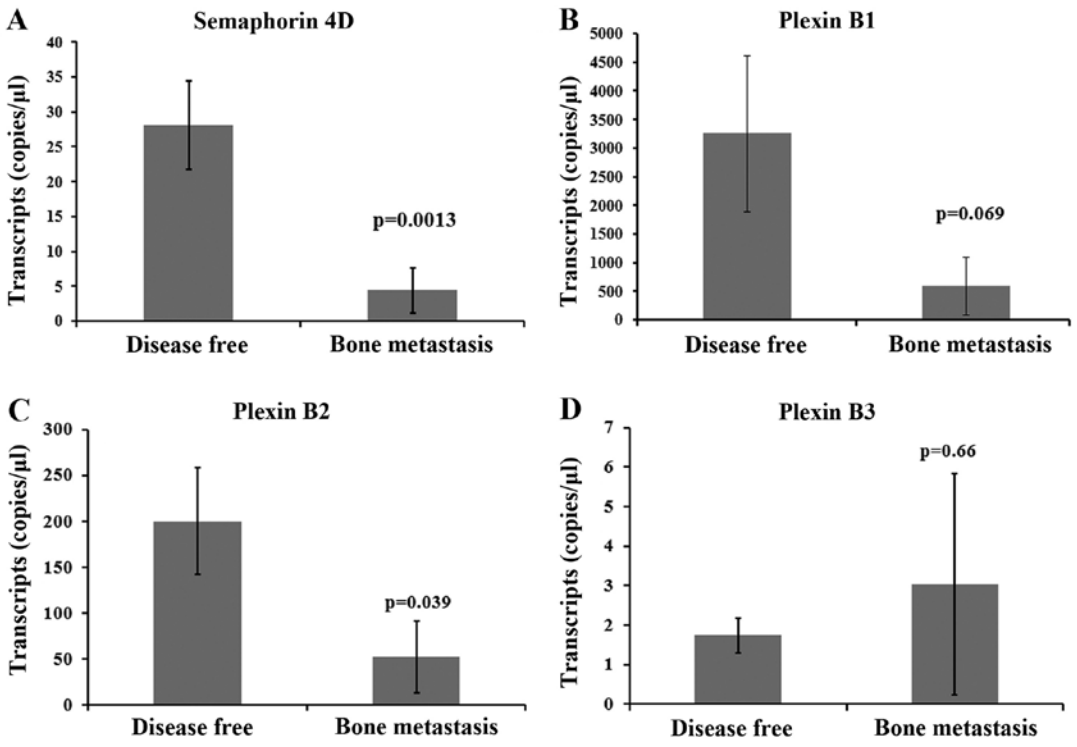

Figure 3. Involvement of Sema4D and the plexin-B family in bone metastasis. (A) Mean transcript values of Sema4D observed in patients suffering from bone metastasis were significantly reduced when compared with disease-free patients (p=0.0013). (B) No significant association of plexin-B1 in relation to bone metastasis was established. (C) Mean transcript values of plexin-B2 observed in patients suffering from bone metastasis were significantly reduced when compared with disease-free patients (p=0.039). (D) No significant association of plexin-B3 in relation to bone metastasis was established.

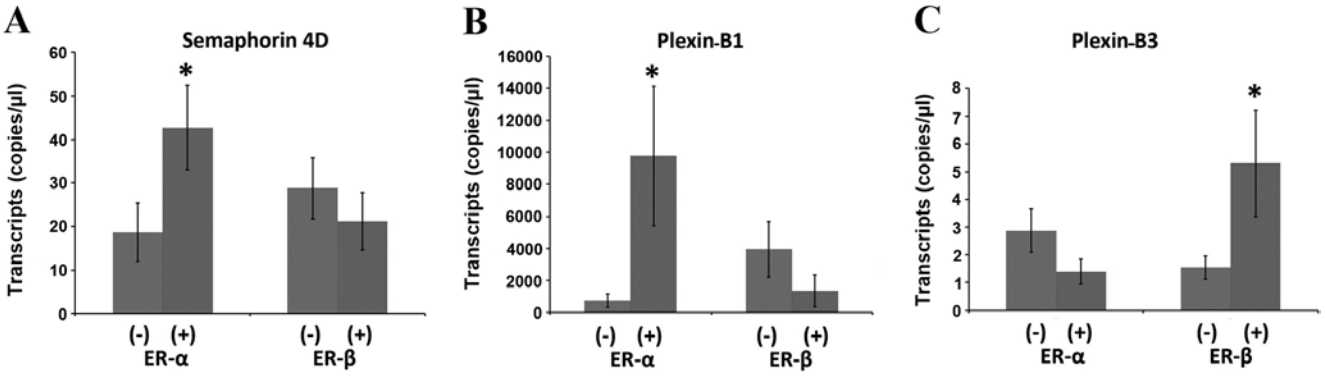

Figure 4. Effect of oestrogen receptors on Sema4D and plexin expression. (A) The ERα-positive breast cancer patients showed an increased Sema4D expression when compared with the ERα-negative patients (p=0.044). No significant association of Sema4D with ERβ was established. (B) Significant association of an increased plexin-B1 expression in the ERα-positive patients was established in comparison to the ERα-negative patients (p=0.05). (C) The ERβ-positive patients showed a significant increase in plexin-B3 expression when compared with the reduced levels observed in the ERβ-negative patients (p=0.01).

in the MDA-MB-231 cells (Fig. 5D). Although these findings were in agreement with cohort observations, this area requires further research to explore the oestrogen-regulated signaling pathways and its effects of semaphorin signalling.

Discussion

The dual role of semaphorins and plexins as oncogene or tumour-suppressor molecules has been previously reported in the literature. As in melanoma, the downregulation of plexin-B1 was strongly associated with cancer progression (36,37), while increased levels of this molecule have been observed in ovarian, prostate and breast cancer (38). In the present study, no significant association of the Sema4D, plexin-B1 and -B2 aberrant expression among normal and diseased patients was observed in the cohort. This result is similar to the findings by Yang *et al*, which show no significant difference in the plexin-B2 protein expression between breast carcinoma and epithelial cells of normal breast tissue (39). In the present study, upregulation of plexin-B3 was identified in breast cancer compared to the normal controls. These findings are also in concordance with the study on gastric cancer patients, the results of which showed an increased expression of plexin-B3 and its ligand (Sema5A) in gastric cancer (40).

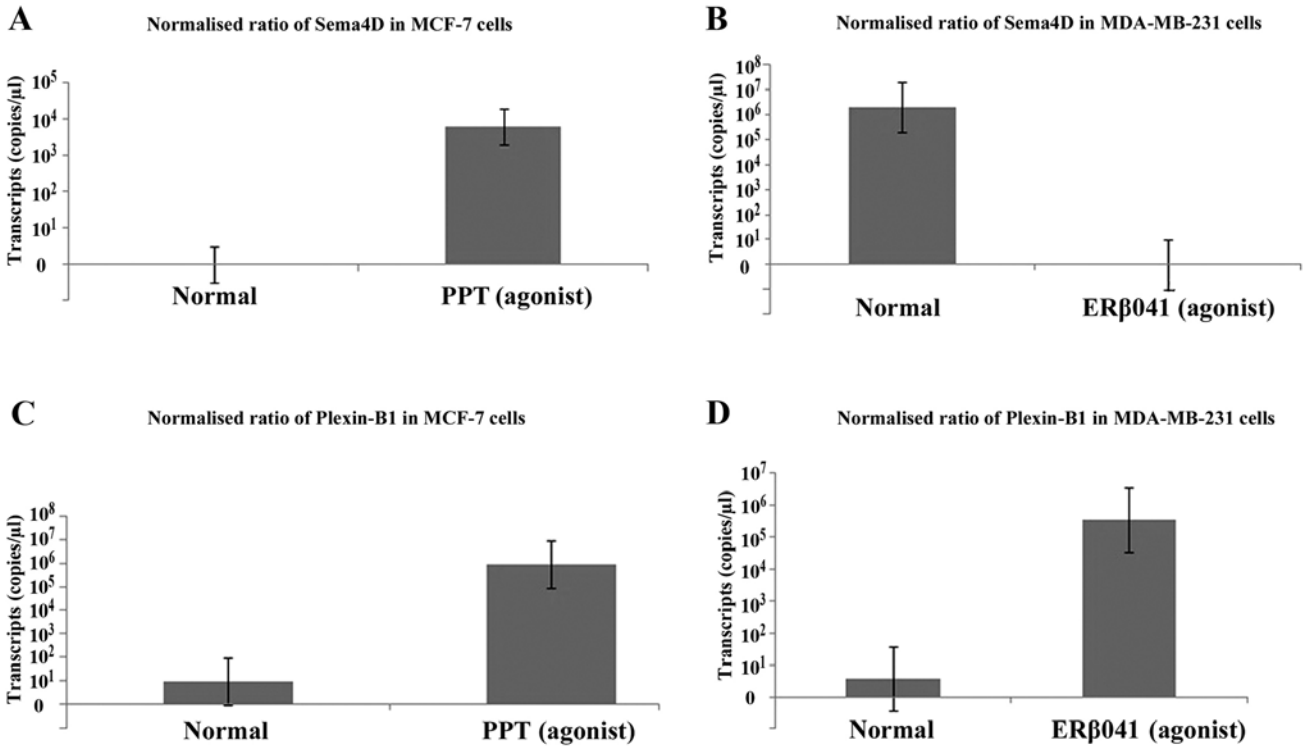

Figure 5. Regulation of Sema4D and plexin-B1 in cell lines using quantitative PCR. (A) Increased expression of Sema4D was observed when wild-type MCF-7 cells were treated with the ERα agonist (PPT). (B) Reduction of Sema4D levels was observed in the MDA-MB-231 wild-type cells upon exposure to an ERβ agonist (ERβ041). (C) Increased expression of plexin-B1 under an ERα agonist (PPT) was observed. (D) Direct correlation of the plexin-B1 expression with an ERβ agonist (ERβ041) is evident. GAPDH was used as an internal control and ratios were generated via normalization. Sema4D, semaphorin 4D.

The reduced expression of Sema4D did not show any significant relationship with tumour cell differentiation in the current cohort. However, the cognate receptor (plexin-B1) showed a strong inverse correlation with tumour cell differentiation. This result is contrary to previous observations reported on breast and ovarian cancer samples. In a previous study, the co-expression of plexin-B1 and Met was associated with worse grading and higher incidences of lymph node metastases (29). These variations are due to i) involvement and influence of other factors interacting with semaphorins and their cognate receptors and ii) heterogeneous tumour orientation. The role of plexin-B1 as a tumour suppressor was observed in melanoma and melanocytes, where B-Raf/MKK/ERK stimulation led to its suppression and tumour progression (36). Thus, plexin-B1-interacting molecules are also noteworthy. In another study, the mammary tumours showing the co-expression of plexin-B2 and Her-2 were characterised as worse staging with higher incidences of lymph node metastases than those that express plexin-B2 alone. However, no significant association of plexin-B2 alone with the TNM stage and grade was observed in these tissues (39). A direct correlation of plexin-B3 with advanced tumour grading in breast cancer cohort was consistent with previous findings. In gastric cancer, a gradual increase in the expression of both receptor and ligand (plexin-B3 and Sema5A) from non-neoplastic mucosa, primary gastric and metastasis was reported (40).

The reduced expression of Sema4D was strongly associated with an unfavourable outcome when compared with the disease-free patients. A study conducted on invasive ductal breast cancers using microarray and qPCR data revealed that 888 genes were significantly ($p \leq 0.05$) differentially expressed between grade I and II tumours. A potentially protective effect of Sema4D, Sema4F and plexin-A2 on benign tumours towards growth and metastatic suppression has been reported (24). Those findings suggest Sema4D putative involvement as a clinical prognostic marker. However, apart from the aforementioned study a completely contrasting feature of Sema4D (acting as oncogene molecule) has also been reported (38,41). As in ovarian cancer, the overexpression of Sema4D together with HIF-1α and VEGF were associated with poor prognosis. An increased expression of Sema4D was also associated with histological grading, stages and lymph node metastasis (41). An increased expression of Sema4D between the cytoplasm and cell surface has also been reported in head and neck squamous cell carcinoma, oral, prostate, breast and lung cancer tissues (38). These disparities in findings are also adequately addressed in the literature. One of the main contributing factors in this regard is that the correlations of these molecules fluctuate strongly when measured over different subsets of patients (42). Loss of plexin-B1 is significantly associated with a worse outcome in patients and the present study was also in concordance with previously published studies (31). A higher expression of plexin-B1 in ER-positive was correlated with the disease-free and overall survival (43).

The expression of Sema4D and plexin-B2 was significantly associated with bone metastasis. Patients having a reduced level of Sema4D and plexin-B2 showed an increased tendency towards bone metastastic progression. This area requires further research to investigate the factors responsible for modulating these effects on bone marrows.

These findings collectively suggest aberrant expression and dysfunctions of these molecules occurring in certain malignancies, including breast cancer, as shown by results of the present study. Conflicting findings from different studies also suggest these molecules may play more complicated roles in cancer regarding the type of cancer, and some other non-clarified subgroups of a particular cancer. For example, in breast cancer the ERs status may be involved in such differences. In the present cohort, a significant increase in Sema4D and plexin-B1 was identified in relation to the ERα-positive tumour patients as compared to the ERα-negative ones. A similar trend regarding the expression of Sema4D and plexin-B1 was also observed in the MCF-7 and MD-MB-231 cancer cell lines following exposure to ERα and ERβ, respectively. These findings are in concordance with previously published studies where increased plexin-B1 levels in ER-positive are correlated with increased disease-free and overall survival (43). A direct expressional correlation of plexin-B1 with ER status was observed in only those cancer cells showing stem cell-like expression status, where proliferative activity was coupled with ER status (31). Thus, the regulation of Sema4D and plexin-B1 influenced by oestrogen receptors is an important domain to determine future therapeutic strategies.

In conclusion, the Sema4D and plexin-B1 reduced levels are associated with breast cancer progression and a poor outcome. Increased plexin-B3 is also a contributory factor for bone metastasis. Oestrogen receptors regulate the expressional profiling of semaphorins and plexins. Involvement of these molecules in bone metastasis and ERs require further investigations to provide a better understanding of the diseases and opportunities to improve personalised treatment according to the molecular signature.

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