Intl. J. Bioinformatics and Biological Sci.: (V. 5 n.1, p. 13-19): June 2017

DOI: 10.5958/2321-7111.2017.00002.6

Integrative Analysis of CXCR4/CXCL12 Axis Gene Expression Alterations in Breast Cancer and its Prognostic Relevance

Helga Jennifer, M., Sneha Mirulalini, G. and Hemamalini, V.*

Department of Bioinformatics, Bharathiar University, Coimbatore, Tamilnadu, India

*Corresponding author: hemamalini@buc.edu.in

ABSTRACT

Cancer metastasis is the major delimiting factor in the failure of therapeutic strategies currently practised and epithelial-mesenchymal transition (EMT) has been observed to be one of the key regulators of metastasis as it confers the invasive phenotype. Chemokines have been shown to be directly involved in mediating the metastatic ability of cancer cells, particularly CXCR4 chemokine receptor. The purpose of the present study was to explore the expression levels of CXCR4/CXCL12 axis in different subtypes of breast cancer using dataset analysis. The mRNA expression levels and genomic alterations of CXCR4 and CXCL12 in different cancers were analyzed via the Oncomine and Cbioportal. In addition, co-expression analysis and clinical survival relevance has been analysed in various datasets. Results of our analysis suggest a significant association of CXCR4/CXCL12 axis in breast cancer as a prognostic biomarker.

Keywords: CXCR4, breast cancer, invasion, survival, co-expression, dataset

Cancer remains one of the most devastating diseases in the world. Reduced survival rate of cancer is mainly due to metastasis, relapse and lack of effective therapies (1). Increasing evidence indicates that tumor micro-environment interactions have a crucial role in tumor initiation and progression (2). Numerous studies indicate that inflammatory environment provided by the chemokines regulates the interaction between tumor cells and stromal cells to create a permissive micro-environment for tumor progression and invasion. The chemokine receptor 4(CXCR4) and its chemokine ligand 12 (CXCL12) are two important factors in the cross-talking between cancer cells and their micro-environment regulating diverse processes in cancer including EMT, invasion, angiogenesis and metastasis (3).

Levels of CXCR4 have been found to increase significantly in cancer cells which enable the tumor cells to acquire resistance to current therapies. Directed metastasis and migration of cancer cells towards target organs is mediated by the SDF1/CXCR4 pathway (4). Studies on leukemia and solid tumors substantiate that CXCR4 signaling events prominently contribute to chemoresistance. Henceforth, targeting the SDF-1/CXCR4 signaling pathway with antagonists has significantly blocked the spreading of tumor cells and development of metastasis in a variety of solid cancers (5).

Chemokine receptor CXCR4 and its ligand CXCL12 expression is found to highly vary across various cancers which strongly suggests its association in cancer progression(6). However, the role of CXCR4 in progression of cancer to metastatic disease is still not fully comprehended. Hence the present study was planned to explore the alterations in mRNA expression of CXCR4 and CXCL12 among cancer

and normal tissues using Oncomine and cBioPortal database. Further we analyzed the CXCR4 expression levels in different subtypes of breast cancer tissues and co-expressed genes pattern were identified. In addition prognostic status was evaluated in different breast cancer cohorts.

Methodology

Oncomine analysis

The Oncomine database was queried for CXCR4 gene in the different datasets based on the following parameters: Cancer type, Cancer versus normal, Drug sensitivity and Perturbations. The analysis was performed using a criterion of a 2 fold change with a p-value of 0.05. In addition, The Cancer Genome Atlas dataset was probed to check the mRNA expression in different breast cancer subtypes. Co-expression analysis was also done to identify sets of genes with synchronous expression patterns. The co-expression profiles of CXCR4 in invasive breast cancer was identified and analysed as the pattern of heat map.

CBioPortal analysis

The frequency of CXCR4 and CXCL12 gene alterations (including mutations, deletions, copy number gains and amplifications) was performed across multiple cancer types using the cBioPortal for Cancer Genomics database (www.cbioportal.org), which contains 166 common cancer studies with the details of almost 46,660 patients.

Prognostic analysis

The KM Plotter which is capable to assess the effect of 54,675 genes on survival using 10,461 cancer samples, including 5,143 breast cancers was used to evaluate the prognostic values of CXCR4 and CXCL12 genes using multivariate analysis for relapse free, Distant metastasis free and overall survival among patients. Cancer patients were split into high and low expression group by the median values of mRNA expression. The stringent value of P-value < 0.01 was considered to indicate a statistically significant result. Survival outcome, HRs, 95% CIs and p-values were summarized and displayed as Kaplan-Meier plots.

RESULTS AND DISCUSSION

In this study, we systematically analyzed the CXCR4 mRNA expression level in various types of cancers using the Oncomine database. We mainly focused on the most common types of breast cancers in relation to the expression frequency to study the role of CXCR4 as a mediator of metastasis. Further, we explored the prognostic values of CXCR4 gene in cancer patients via the Kaplan-Meier Plotter database.

CXCR4 Expression across various cancers

To address the CXCR4 mRNA expression differences between tumor and normal tissues in multiple cancers, we performed an analysis using the Oncomine database (7). As shown in Fig. 1, the database contained a total of 449 unique analyses.

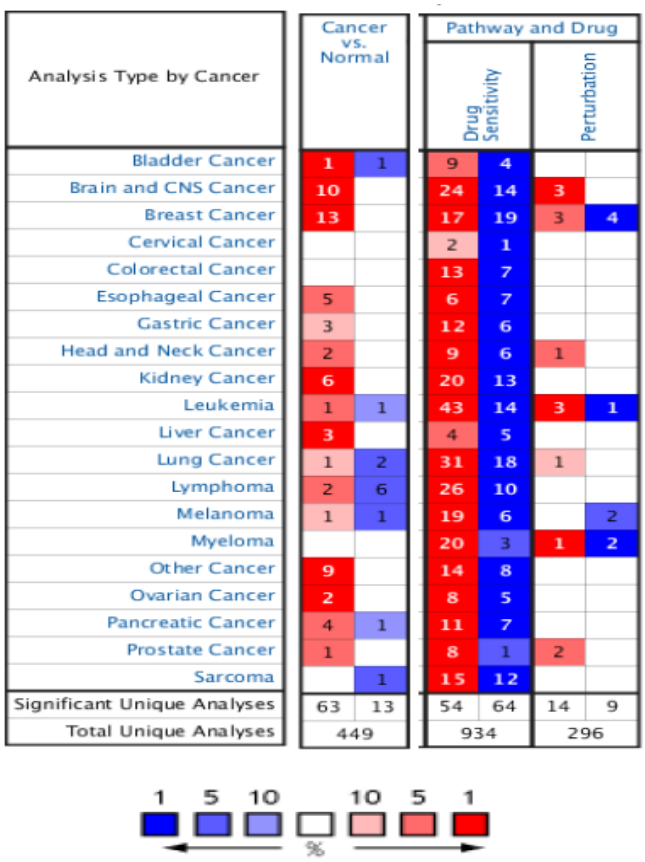


Fig. 1: Differential expression of CXCR4 gene in various cancers in comparison with normal,Data shown based on<0.05 threshold P-value changes set by Oncomine. Over-expression or under-expression

in the top 1, 5 and 10% are colour-coded according to the legend. The "red cells" represents CXCR4 over expression and the "blue cells" represent CXCR4 under expression. The levels of expression are based on the gene rank percentile.

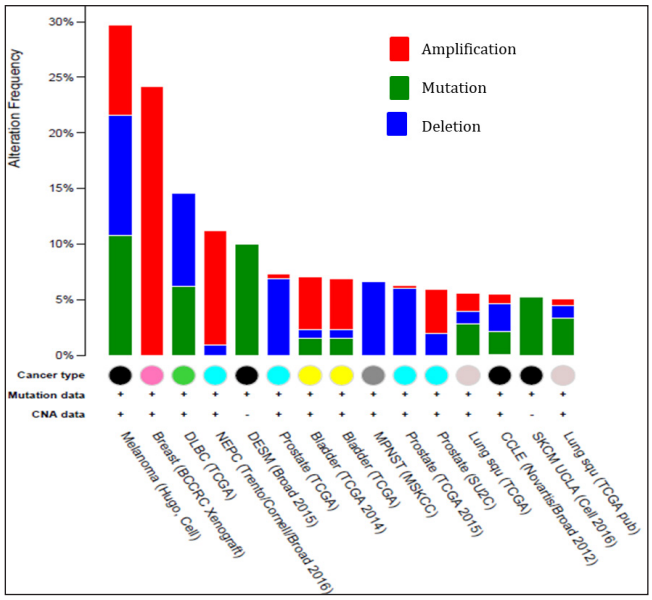
In 76 studies, CXCR4 showed significant difference among all the known cancers except few, 13 cohorts revealed higher expression levels in tumor compared to normal tissues. Since, CXCR4 is supposed to be the mediator of cancer metastasis, we assessed the expression levels based on drug sensitivity and pathway perturbations. All cancer types showed a marked response to chemotherapeutic drugs but there was a significant marked increase in the CXCR4 expression following chemotherapy as indicated in brain, breast, leukemia and lung cancer demonstrating pathway perturbations occurring in these cancers leading to relapse of tumors.

Further analysis on the expression levels of CXCR4 demonstrated significant over expression across various carcinomas. Hence using the aforesaid stringent criteria analysis, CXCR4 expression across the analyzed cancers for Drug sensitivity has been shown in the Fig. 1. There is a significant change in the expression of CXCR4 following treatment indicating pathway perturbations in breast, brain and other cancers which correlates tissue specific development of secondary tumors.

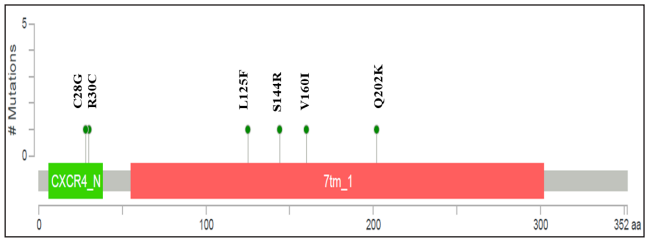
We explored the genomic alternations of CXCR4 and CXCL12 according to the cBioPortal database containing the sequencing results in TCGA (8). CXCL12 gene was amplified in several types of human cancer, with an amplification of 24% in breast cancer and 10% in prostate cancer (Fig. 2A). However, we noticed that there was no amplification of CXCR4 rather only missense mutations existed in a wide range of cancers.

Survival analysis

The prognostic value of SDF1/CXCR4 gene in breast cancer patients using "the Kaplan-Meier plotter" database was analyzed, which contains updated gene expression data and survival information from a total of 5143 breast cancer patients (9). When all the patients were separated from middle and defined as high and low expression groups, the results revealed that patients with high CXCR4 expression experienced a short relapse-free survival compared with patients of low CXCR4 expression, with a 27% increase in the risk of recurrence (Fig. 3). Notably, patients with high CXCR4 expression had a markedly poor overall survival (p = 0.0002) and Distant metastasis free survival (p = 0.002) compared to patients with low expression.



(A)



(B)

Fig. 2: CXCR4 is up-regulated in breast cancer tissues and predicts poor prognosis. (A) Graph indicates the frequency of genomic alternations in CXCR4/CXCL12 gene various cancers according to the cBioPortal database. (B) Diagrammatic representation of the mutations reported in the CXCR4 gene.

CXCR4 Expression in breast cancer

Oncomine database has been explored to assess the expression of CXCR4 in different breast cancer

Table 1: Breast cancer dataset for CXCR4 gene

Dataset	Normal cases	Tumor Cases	Fold Change	t-test	p-value
Richardson	Breast (7)	Ductal Breast Carcinoma (40)	3.048	6.405	8.43E-7
Finak	Breast (6)	Invasive Breast Carcinoma (53)	7.230	16.964	8.95E-19
Gluck	Breast (4)	Invasive Breast Carcinoma (154)	2.873	7.574	6.63E-4
Karnoub	Breast (15)	Invasive Ductal Breast Carcinoma(7)	2.659	5.034	3.27E-5
Curtis	Breast (144)	Invasive Ductal and Invasive Lobular Breast Carcinoma (90)	2.226	13.117	7.04E-28

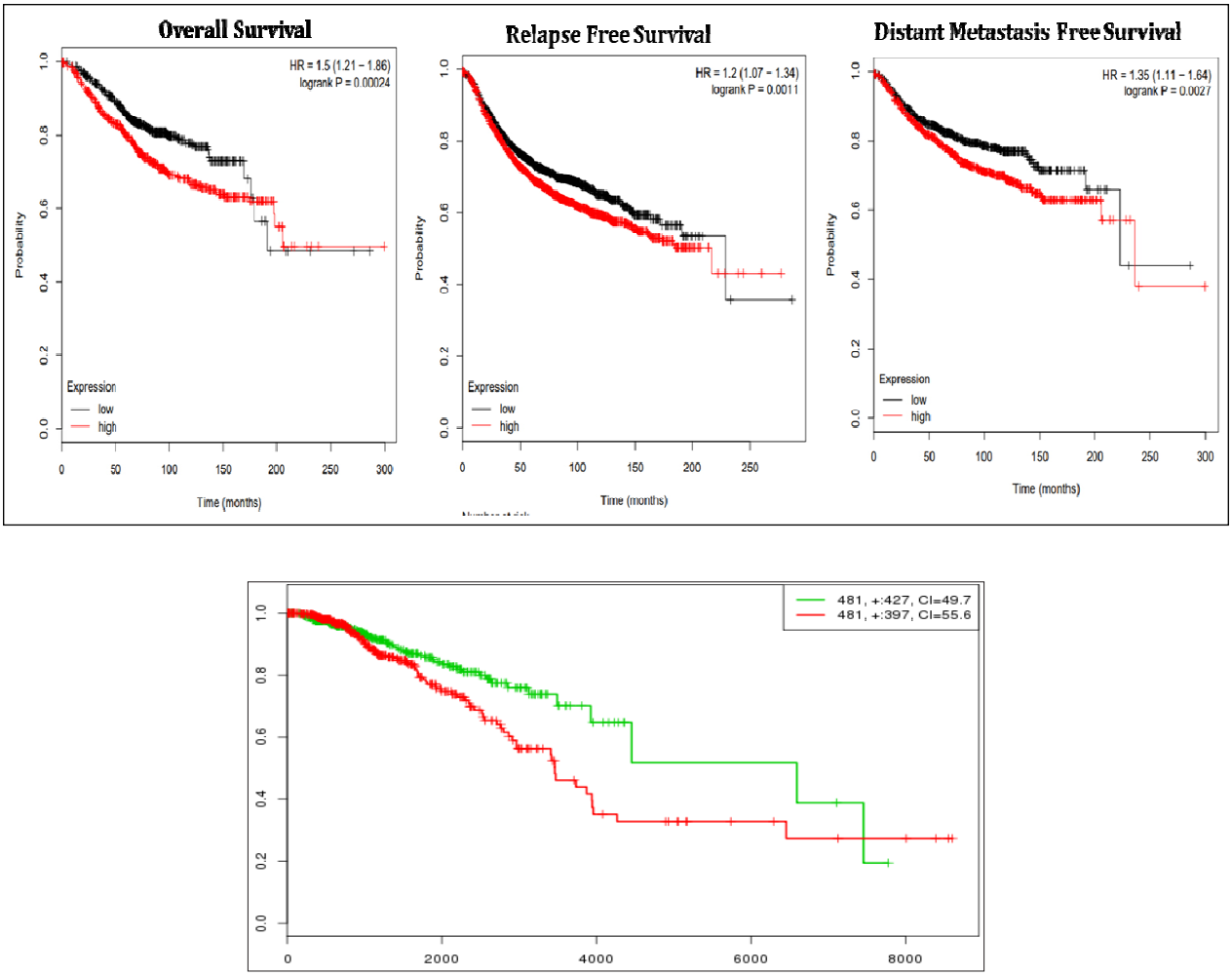
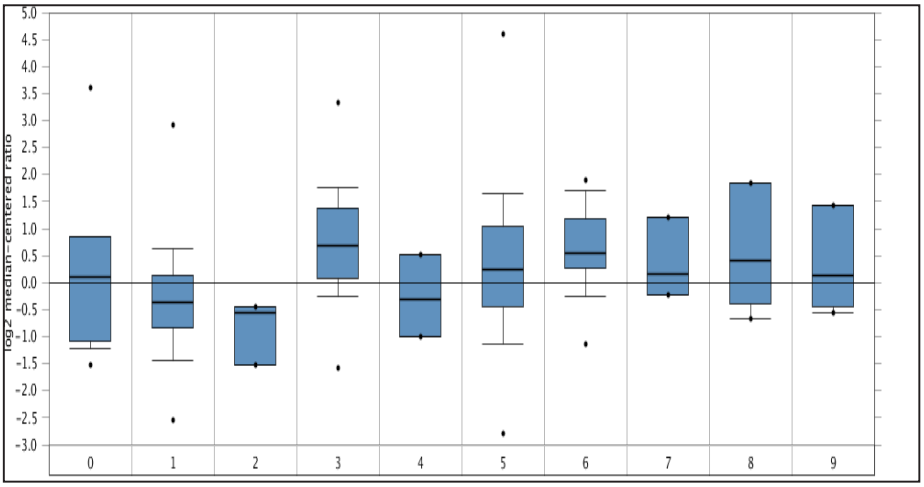


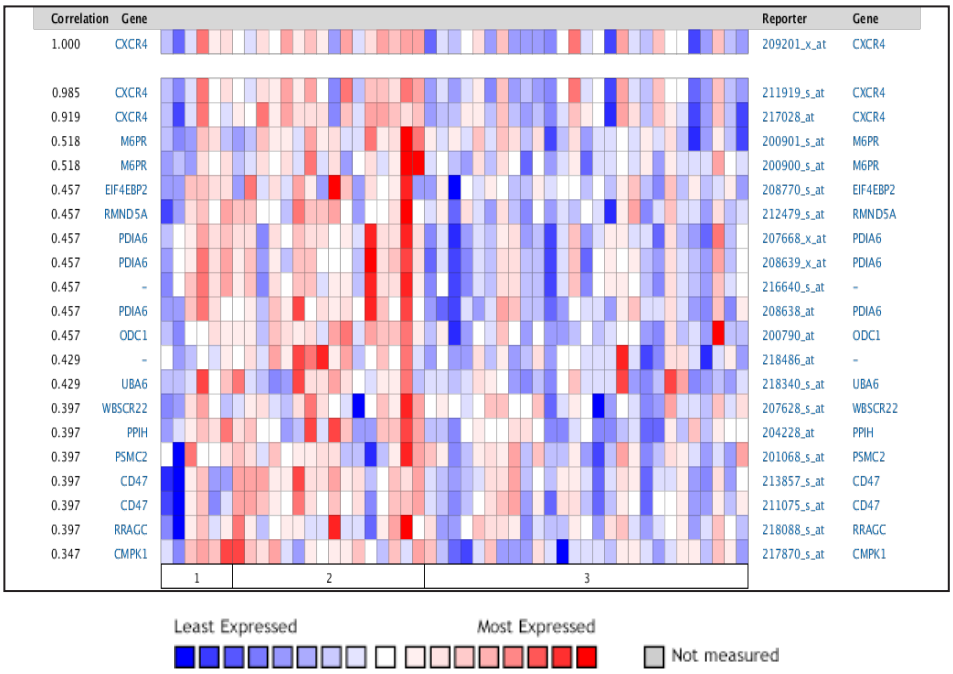
Fig. 3: Survival Analyses of CXCR4/CXCL12 gene in breast cancer. The Kaplan–Meier analysis indicated that highexpression of CXCR4 predictedworse overall survival and poor Relapse-free survival and Distant metastasis free survival. Recent TCGA data on invasive breast cancer shows significant prognostic correlation with CXCR4.

subtypes. A total of 5 datasets [Finak (10), Karnoub (11), Curtis (12), Gluck (13) and Richardson (14) cohorts] were involved in the analysis which had a relative comparison with the normal types. We found that CXCR4 levels were significantly up-regulated in human breast cancer tissues compared with that



1. Breast (61) 2. Intraductal Cribriform Breast Adenocarcinoma (3) 3. Invasive Breast Carcinoma (76) 4. Invasive Ductal and Lobular Carcinoma (3) 5. Invasive Ductal Breast Carcinoma (389) 6. Invasive Lobular Breast Carcinoma (36)

Fig. 4: CXCR4 Expression in TCGA Breast cancer sub types – the numbers in bracket indicate the numbers of cases considered for the study.



1. Apocrine Breast Carcinoma (6)
2. Basal-Like Subtype of Invasive Breast Carcinoma (16)
3. Luminal-Like Subtype of Invasive Breast Carcinoma (27)

Fig. 5: CXCR4 co-expression analysis in breast dataset. The indicated genes are co-expressed along with CXCR4 in the basal and luminal subtypes of breast cancer. The Basal subtype displays a significant high expression of genes compared to Luminal type.

in non-cancer tissues (Table 1). We further assessed the CXCR4 levels of different cancer subtypes in 593 breast cancer tissues according to the TCGA database. Compared to normal breast, CXCR4 is significantly over expressed in subtypes of breast cancers such as Ductal, Lobular and Medullary. There was a significant increase in the CXCR4 expression in invasive or metastasized groups compared to primary tumors which probably indicates the predominant role of CXCR4 in mediating EMT for cancer progression. All of the statistically significant results were summarized in Fig.4.

Co-expression analysis of CXCR4 gene in the Oncomine Farmers breast dataset showed that the Basal-like subtype showed a higher coexpression of genes compared to Luminal subtype (Fig. 5) indicating the invasive ability of basal subtype and poorer prognosis.

In summary, CXCR4 gene was amplified in several types of cancers, especially in breast cancer and prostate cancer, as the frequency of amplification was above 10% (Fig. 2A). Also, we determined the significance of CXCR4 expression in different subtypes of breast cancer. We found that high CXCR4 was closely correlated with induction of EMT and invasion(15). Importantly, CXCR4 was significantly associated with low survival rate in both overall and recurrence-free survival. Thus our analysis suggested that CXCR4 was involved in the progression and relapse of breast cancer (16).

CONCLUSION

We comprehensively analyzed the mRNA expression levels and prognostic values of CXCR4/CXCL12 axis in most common cancers. CXCR4, exhibited significant expression differences between cancer and normal tissue groups in defined cancers. Furthermore, we put forward that CXCR4 could act as a prognostic biomarker for detection of invasive breast cancer. However, we concentrated on only the mRNA expression levels and the prognostic values of CXCR4, neither their protein expression levels nor signaling pathways were analyzed. More cohort studies are needed to validate the prognostic values, and further studies should be carried out to explore the underlying molecular mechanisms mediated by CXCR4.

REFERENCES

Curtis C, Shah SP, Chin SF, Turashvili G, Rueda OM, Dunning MJ, Speed D, Lynch AG, Samarajiwa S, Yuan Y, Gräf S, Ha G, Haffari G, Bashashati A, Russell R, McKinney S, Group M, Langerød A, Green A, Provenzano E, Wishart G, Pinder S, Watson P, Markowetz F, Murphy L, Ellis I, Purushotham A, Børresen-Dale A-L, Brenton JD, Tavaré S, Caldas C, Aparicio S. 2012. The genomic and transcriptomic architecture of 2,000 breast tumours reveals novel subgroups. *Nature*, **486**: 346-352.

Domanska UM, Kruizinga RC, Nagengast WB, Timmer-Bosscha H, Huls G, de Vries EG, Walenkamp AM. 2013. Jan; A review on CXCR4/CXCL12 axis in oncology: no place to hide. *Eur J Cancer.* **49**(1): 219-30.

Domanska UM, Kruizinga RC, Nagengast WB, Timmer-Bosscha H, Huls G, de Vries EG, Walenkamp AM. 2013: Jan. A review on CXCR4/CXCL12 axis in oncology: no place to hide. *Eur J Cancer.* **49**(1): 219-30.

Finak G, Bertos N, Pepin F, Sadekova S, Souleimanova M, Zhao H, *et al*. 2008. Stromal gene expression predicts clinical outcome in breast cancer. *Nature medicine,* **14**(5): 518-27.

Furusato, B. and Rhim, J.S. CXCR4 and Cancer. A.M. 2009. Fulton (ed.), Chemokine Receptors in Cancer, Cancer Drug Discovery and Development, Humana Press, a part of Springer Science Business Media, LLC.

Gao J, Aksoy BA, Dogrusoz U, Dresdner G, Gross B, Sumer SO, *et al*. 2013. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. *Sci Signal*. 6:11.

Glück S., Germond C., Lopez P., *et al*. 1998. A phase I trial of high-dose paclitaxel, cyclophosphamide and mitoxantrone with autologous blood stem cell support for the treatment of metastatic breast cancer. *European Journal of Cancer.* **34**(7): 1008–1014.

Gyorffy B, Lanczky A, Eklund AC, Denkert C, Budczies J, Li Q, *et al*. 2010. An online survival analysis tool to rapidly assess the effect of 22,277 genes on breast cancer prognosis using microarray data of 1,809 patients. *Breast Cancer Research and Treatment*, **23**(3): 725±31.

Hanahan, D. and R.A. Weinberg, 2000. *The hallmarks of cancer. Cell,* **100**(1): 57-70.

Kalluri, R. and R.A. Weinberg, 2009. The basics of epithelial-mesenchymal transition. *J Clin Invest,* **119**(6): 1420-8.

Karnoub AE, Dash AB, Vo AP, Sullivan A *et al*. 2007 Oct 4. Mesenchymal stem cells within tumour stromapromote breast cancer metastasis. *Nature,* **449**(7162): 557-63.

Kim, M., *et al*. 2010. CXCR4 signaling regulates metastasis of chemoresistant melanoma cells by a lymphatic metastatic niche. *Cancer Res,* **70**(24): 10411-21.

Liu, T. *et al*. 2015. Effectiveness of AMD3100 in treatment of leukemia and solid tumors: from original discovery to use in current clinical practice. *Exp. Hematol. Oncol.,* **5**: 19.

Ma, X.J., Dahiya S., Richardson E., Erlander M. and Sgroi D. C. 2009. Gene expression profiling of the tumor micro-environment during breast cancer progression. *Breast Cancer Research*, **11**(1, article R7).

Rhodes, D.R., Kalyana-Sundaram, S., Mahavisno, V., Varambally, R., Yu, J., Briggs, B.B., Barrette, T.R., Anstet, M.J., Kincead-Beal, C., Kulkarni, P., Varambally, S., Ghosh, D., and Chinnaiyan, A.M. 2007. Oncomine 3.0: genes, pathways, and networks in a collection of 18,000 cancer gene expression profiles. *Neoplasia,* **9**: 166–180.

Xu, C. *et al*. 2015. CXCR4 in breast cancer: oncogenic role and therapeutic targeting. *Drug Des Devel Ther.,* **9**: 4953-64.