Journal of
Cancer Science & Therapy

Wei et al., J Cancer Sci Ther 2012, 4.9
DOI: 10.4172/1948-5956.1000158

Research Article Open Access

Evaluating the Prognostic Significance of *FBXW7* Expression Level in Human Breast Cancer by a Meta-analysis of Transcriptional Profiles

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Abstract

The tumor suppressor gene *FBXW7* is mutated in numerous types of human cancers leading to loss of its function and/or expression. However the clinic significance of *FBXW7* alterations remains largely unknown. Here, we carried out a meta-analysis of 10 gene expression microarray studies for a total 1900 patients of breast cancer with clinic information to evaluate the prognostic impact of *FBXW7* mRNA expression. The *FBXW7* mRNA levels significantly reduced in breast cancer compared to normal tissues. In addition, significant difference in the *FBXW7* mRNA levels was found among molecular subtypes (normal-like, luminal A, luminal B, ERBB2 and basal). ERBB2 and basal tumors had significantly lower average *FBXW7* mRNA level than normal-like tumors, whereas luminal A and B tumors have the lowest average *FBXW7* mRNA level. The patients with higher *FBXW7* mRNA level significantly increased disease-free survival, particularly in the group of patients with ER negative and basal subtype tumors. Moreover, higher *FBXW7* mRNA level also significantly increased overall survival in the patients with ER negative tumors. But we strikingly found opposite effect of *FBXW7* expression on overall survival in different subtypes. The patients with higher *FBXW7* mRNA level significantly decreased overall survival in normal-like subtype while the patients with higher *FBXW7* mRNA level significantly increased overall survival in ERBB2 and Basal subtype. Taken together, our results suggest that *FBXW7* mRNA levels were a prognostic factor for disease-free and overall survival according to ER status and molecular subtypes.

Keywords: Breast cancer; *FBXW7;* Meta-analysis; Transcriptional profile; Prognosis

Introduction

Breast cancer is the most frequent malignancy in women worldwide [1,2] and has had a major impact on both health and the economy of the United States [1]. While some improvements have been made in diagnosis and treatment of breast cancer, the prognosis and survival for most patients has not dramatically changed. Many women who are at low risk of disease progression are subjected to unnecessary, aggressive therapy from which they are unlikely to benefit, because of inadequacy in reliable predictors of outcome. Many patients in the poor prognosis category either fail to respond to available treatments, or show transient responses followed by development of drug resistance. Furthermore, breast tumors display remarkable biological and clinical heterogeneity. Developing a better understanding of the molecular basis of such heterogeneity is urgently required for individualized treatment for patients based on molecular profiling.

Several endpoints including overall survival (OS) and disease-free survival (DFS) have been used to assess the clinic benefit of cancer treatments. OS has long been the gold standard primary endpoint for the demonstration of clinical benefit. OS denotes the proportion of patients remain alive at a specified time after treatment, which takes into account death due to any cause including both related and unrelated to the cancer. However, DFS is increasingly being used as the primary endpoint of most studies testing the benefits of potential cancer therapies since DFS is observed earlier than OS and is statistically sensitive to real treatment benefit. DFS denotes the proportion of patients remain free of disease at a specified time after treatment.

F-box and WD repeat domain containing 7 (FBXW7) protein encodes a substrate adaptor for an E3 SCF ubiquitin ligase complex and negatively regulates the abundance of different oncoproteins, including c-Myc [3,4], c-Jun [5], cyclin E [6,7], different members of the Notch family [8,9], Aurora-A [10-12], mTor [13,14], KLF5 [15,16], and MCL-1 [17,18]. These observations indicate that FBXW7 lies at the nexus of many pathways which control cell growth, cell differentiation, and tumorigenesis. *FBXW7* as a human tumor suppressor gene is further supported by the discovery of *FBXW7* gene mutations in cancers from a wide spectrum of human tissues with overall 6% point mutation frequency [19,20]. A recent study showed that breast cancer patients with low *FBXW7* mRNA levels had poorer prognoses than those with high expression when analyzing breast cancer-specific survival, whereas *FBXW7* mRNA levels did not affect DSF [21]. However, the clinical significance of *FBXW7* alteration in human cancer still remains largely unknown. In this study, we conducted a meta-analysis of pooled transcriptional data from 10 studies to evaluate the prognostic impact of *FBXW7* expression level in human breast cancer by assessing its association with DFS and OS in further depth by considering the heterogeneity of breast cancer.

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Received July 24, 2012; **Accepted** August 29, 2012; **Published** August 31, 2012

Citation: Wei G, Wang Y, Zhang P, Lu J, Mao JH (2012) Evaluating the Prognostic Significance of *FBXW7* Expression Level in Human Breast Cancer by a Meta-analysis of Transcriptional Profiles. J Cancer Sci Ther 4: 299-305. doi:10.4172/1948-5956.1000158

Materials and Methods

Transcriptional datasets used in study

We used 2 previously published transcriptional profiling datasets that contained both normal and breast cancer samples and 10 breast cancer datasets that included clinical and gene expression data (Table 1). The normal and primary human breast tumor samples in these datasets had been profiled with an Affymetrix microarray assay (either HG-U133A or HG U133 Plus 2.0) or Agilent oligo microarray [22-33] (Table 1). The process data from GEO website were downloaded for analysis. In total, data on DFS and OS were available for 1900 and 889 of 1935 patients, respectively, in 10 datasets (Table 1).

Probe 218751_s_at and 19539 (Genebank NM_018315) were used to measure *FBXW7* mRNA expression in Affymetrix and Agilent GeneChip, respectively. In each dataset, a sample s_i in the set S was defined as "FBXW7 Low," "FBXW7 Intermediate", or "FBXW7 High" using the rule:

If $s_i \leq$ (mean [S]-0.5×standard deviation [S]), assign Low.

If $s_i \geq$ (mean [S]+0.5×standard deviation [S]), assign High.

Otherwise, assign Intermediate. The same rule has been used in our previous study of PER3 [34].

This method allowed us to compare relative *FBXW7* mRNA expression levels across the different platforms of microarrays and all datasets fused as a single group of patients.

Statistical analysis

The difference in *FBXW7* mRNA expression levels between normal and breast cancers was analyzed by Mann-Whitney U. Kruskal-Wallis test was used to assess the difference in *FBXW7* mRNA expression levels among molecular subtypes. Kaplan-Meier plots were constructed and a long-rank test was used to determine differences among disease-free and overall survival according to *FBXW7* mRNA levels or molecular subtypes. All analyses were performed by SPSS 11.5.0 for Windows. A two-tailed p-value of less than 0.05 was considered to indicate statistical significance.

Dataset*	GEO access number or web location	Number of patients	% DFS data available	% OS data available	References
1	GSE1456	159	100	100	[22]
2	GSE2603	99	82.8	0	[23]
3	GSE6532	327	96.0	0	[24]
4	GSE3494	108[&]	100	100	[25]
5	GSE7390	198	100	100	[26]
6	GSE11121	197	100	0	[27]
7	GSE12093	136	100	0	[28]
8	GSE2034	286	100	0	[29]
9	ArrayExpress:E-TABM-158	130	99.2	99.2	[30]
10	NKI (http://microarray-pubs.stanford.edu/wound_NKI/)	295	100	100	[31]
11	GSE17080	185	0	0	[32]
12	GSE3744	47	0	0	[33]

*Dataset 1 to 9, 11 and 12 are profiled using Affymetrix microarray, Dataset 11 using Agilent Oligo microarray. Dataset 1-10 are used to evaluate prognostic impact of *FBXW7* expression. Dataset 1-10 was used for analysis of disease-free survival in Figure 2, 3, 5 and 6. Dataset 1, 4, 5, 9 and 10 was used for analysis of overall survival in Figure 2, 3, 5 and 6. Dataset 11 and 12 contains both normal and breast cancer samples.

[&]There are total 251 patents in GSE3494, 143 of them overlapped with GSE6532 were removed.

Table 1: Information of gene expression datasets used in this study.

Results

We first examined *FBXW7* gene expression in two datasets that contained both normal and breast cancer samples (Dataset 11 and 12 in Table 1). The levels of *FBXW7* mRNA in invasive ductal carcinomas (IDC) were statistically significantly lower than these in the normal breast ducts (p=1.09E-13) (Figure 1a). In other dataset it was also found that the level of *FBXW7* mRNA was significantly lower in breast cancers than in normal breast tissue (p=0.0013) (Figure 1b). These findings were consistent with that *FBXW7* plays a tumor suppressive role in breast cancer.

We next examined the association of *FBXW7* mRNA levels with disease-free survival in 1900 breast tumor patients taken from 10 publicly available datasets (Table 1). To do so, we first divided the patients into different subgroups ("FBXW7 High", "FBXW7 Intermediate", and "FBXW7 Low") according to *FBXW7* mRNA levels in each dataset (details see Materials and Methods) and then pooled all datasets as a single group of patients. The DFS curves for all patients in each subgroup were shown in Figures 2a. Patients with high *FBXW7* mRNA levels ("FBXW7 High") significantly increased DFS than those with Intermediate ("FBXW7 Intermediate") (p=0.019) or low ("FBXW7 Low") (p=0.025) *FBXW7* mRNA levels (p=0.037 obtained by long rank test among three groups) (Figure 2a). There is no difference in DFS curves between *FBXW7* Intermediate and Low groups, suggesting there is no dose-dependent effect of *FBXW7* expression on DFS. Surprisingly, there is no significant effect of FBXW7 mRNA levels on OS (p=0.35) (Figure 2b).

Estrogen receptor (ER) status in breast cancer is an important predictor of recurrence and greatly influences treatment regimens. If low expression of *FBXW7* mRNA segregates with ER status, any effect of low *FBXW7* expression could be confounded with the effect of ER status. We therefore performed a subset analysis of FBXW7 in ER-positive and ER-negative tumors. Lower levels of FBXW7 expression were significantly associated with shorter DFS and OS (p=0.038 and 0.010 respectively) in patients with ER-negative, but not ER-positive tumors (Figure 3).

The molecular subtype of human breast cancer is another important prognostic factor. We next asked whether stratifying tumors according to their molecular subtype could reveal additional information of *FBXW7* expression associated with breast cancer. Therefore the tumors were assigned using a nearest centroid classifier [35,36]. A subtype was only assigned if correlation with a target class was above 0.1. This resulted in samples assigned as normal-like (n=265), luminal A (n=550), luminal B (n=342), ERBB2 (n=193), basal (n=382), or Unclassified (n=282). The levels of *FBXW7* expression were significantly different among different molecular subtypes (Figure 4). It was found surprisingly that luminal A tumors have the lowest average level of *FBXW7* whereas the normal-like tumors have the highest average level of *FBXW7* in most of datasets (Figure 4). We then performed a subset analysis of *FBXW7* in each molecular subtype tumors. Of these groups, only in basal subtype, the patients with *FBXW7* High significantly increased DFS (p=0.040) (Figure 5). There was a striking effect of *FBXW7* expression on OS among different molecular subtypes (Figure 6). In normal-like subtype, the patients with *FBXW7* High significantly decreased OS (p=0.044

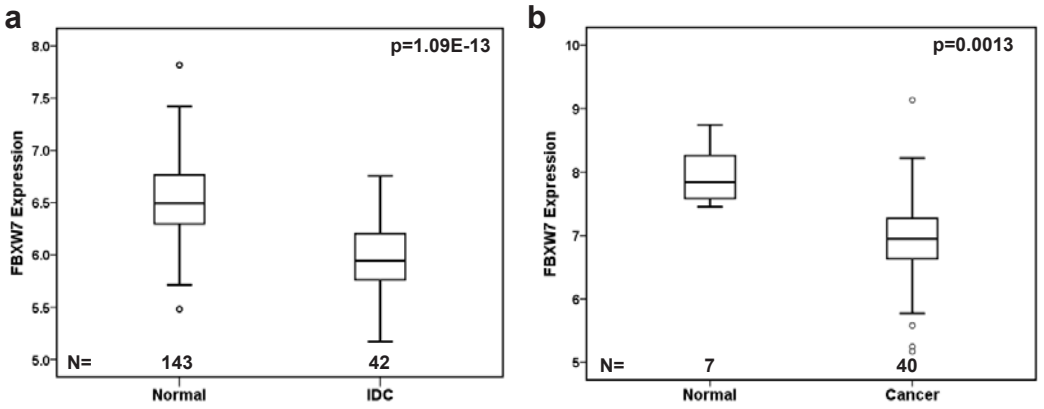

Figure 1: *FBXW7* expression level in human breast cancer and normal tissues. *FBXW7* mRNA expression is assessed by Affymetrix microarray. *FBXW7* expression is measured as $\log_2$ (probe intensities). In both dataset GSE10780 (dataset 11 in Table 1) (a) and GSE3844 (dataset 12 in Table 1) (b), *FBXW7* mRNA expression level is significantly reduced in breast tumors in comparison to normal breast tissues.

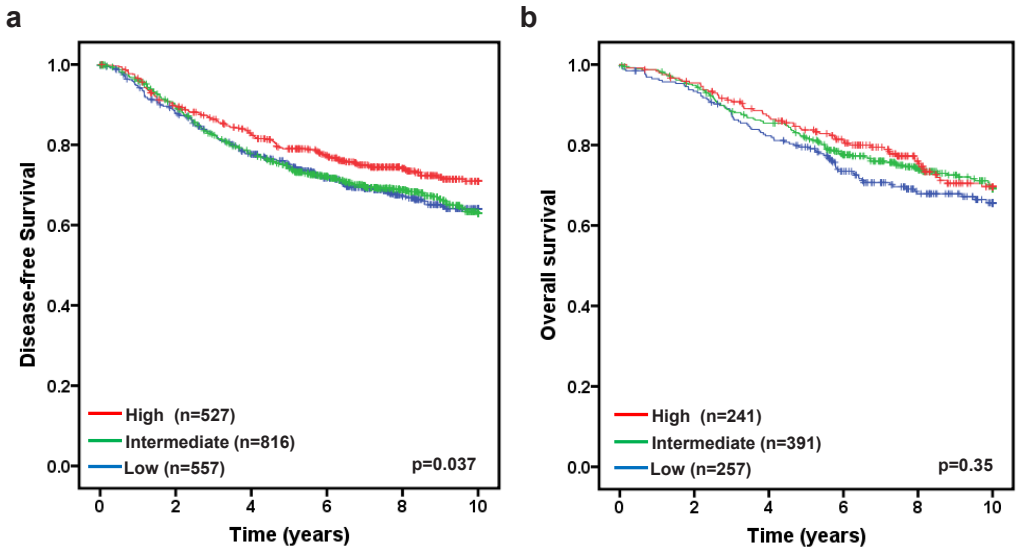

Figure 2: Evaluating the prognostic impact of *FBXW7* mRNA expression on disease-free and overall survival. Kaplan-Meier survival curves for breast cancer patients according to tumor expression of *FBXW7* are presented. The patients from each cohort were divided into a group with High, Intermediate, or Low level of *FBXW7* expression. The p values shown were obtained from a long-rank test among three groups. (a) Association of *FBXW7* expression with disease-free survival. Dataset 1 to 10 in Table 1 were included in the analysis. (b) Association of *FBXW7* expression with overall survival. Dataset 1, 4, 5, 9 and 10 in Table 1 were included. In other datasets were excluded due to that the information of overall survival was missing.

obtained by long rank test among three groups) (Figure 6a), while in ERBB2 and basal subtype, the patients with *FBXW7* High significantly increased OS (p=0.003 and 0.049, respectively) (Figure 6d and e).

Discussion

There is a large amount of evidences showing that *FBXW7* is a tumor suppressor gene in human cancer (review see [20]. Searching distribution of somatic mutations in *FBXW7* in Catalogue of Somatic Mutation In Cancer (COSMIC) (http://www.sanger.ac.uk/genetics/CGP/cosmic/ we found that there is about 1% of *FBXW7* point mutation in breast cancer. However deletions of chromosome 4q31, on which *FBXW7* is located, are found in more than 30% of breast cancer cell lines and primary cancers [30,37]. Since loss of only one copy of *FBXW7* can have a substantial effect on tumor development, it is likely that the overall impact of this gene in human breast cancer is greater. Consistent with these findings, this study showed that the *FBXW7* expression levels were significantly reduced in breast cancer compared to the normal breast tissues in both datasets. Intriguingly, luminal A tumors displayed the lowest average level of *FBXW7* expression whereas normal-like tumors displayed the highest average level of *FBXW7* expression.

In this meta-analysis study, we used the public transcriptional profiles to evaluate the prognostic value of *FBXW7* expression in breast cancer. We found that the lower levels of *FBXW7* were significantly associated with shorter DFS, particularly in patients with ER-negative and basal subtype tumors. With no effect was seen in patients with ER-positive and other subtype tumors, we conclude that the association between *FBXW7* expression and DFS in the complete patient sample set was driven by the ER-negative and basal subtype tumors. The recent study by Ibusuki et al. [21] found no significant difference in DFS between the patients with low and high *FBXW7* expression, which is

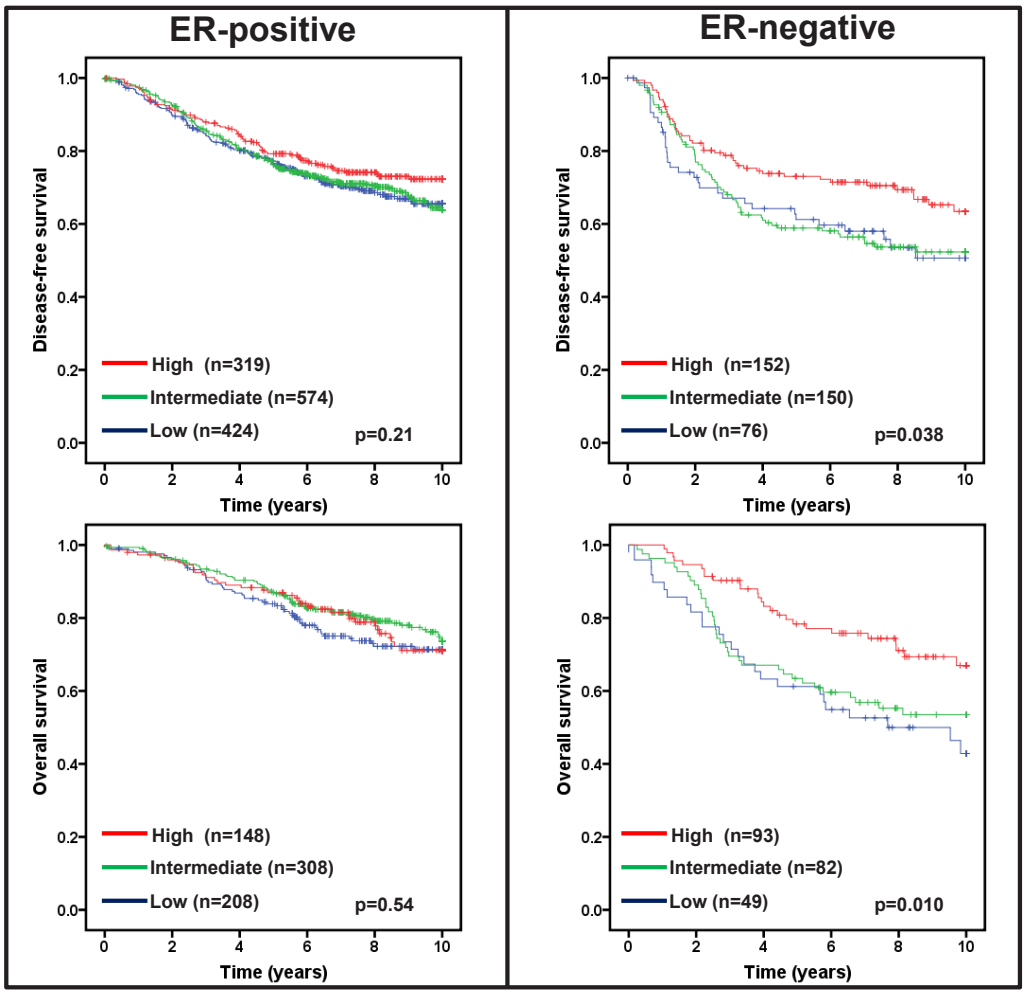

Figure 3: Effect of *FBXW7* expression levels on DFS and OS according to ER status. Kaplan-Meier estimates of DFS and OS according to the *FBXW7* expression are presented. The p values shown were obtained from a long-rank test among three groups.

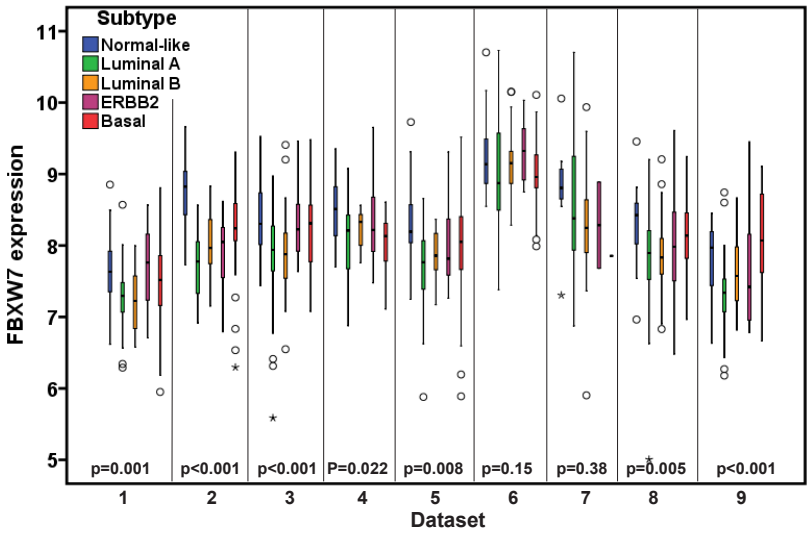

Figure 4: There are significant differences in *FBXW7* expression among molecular subtypes. The p-values obtained from Kruskal-Wallis test by comparing the difference in *FBXW7* expression among subtypes.

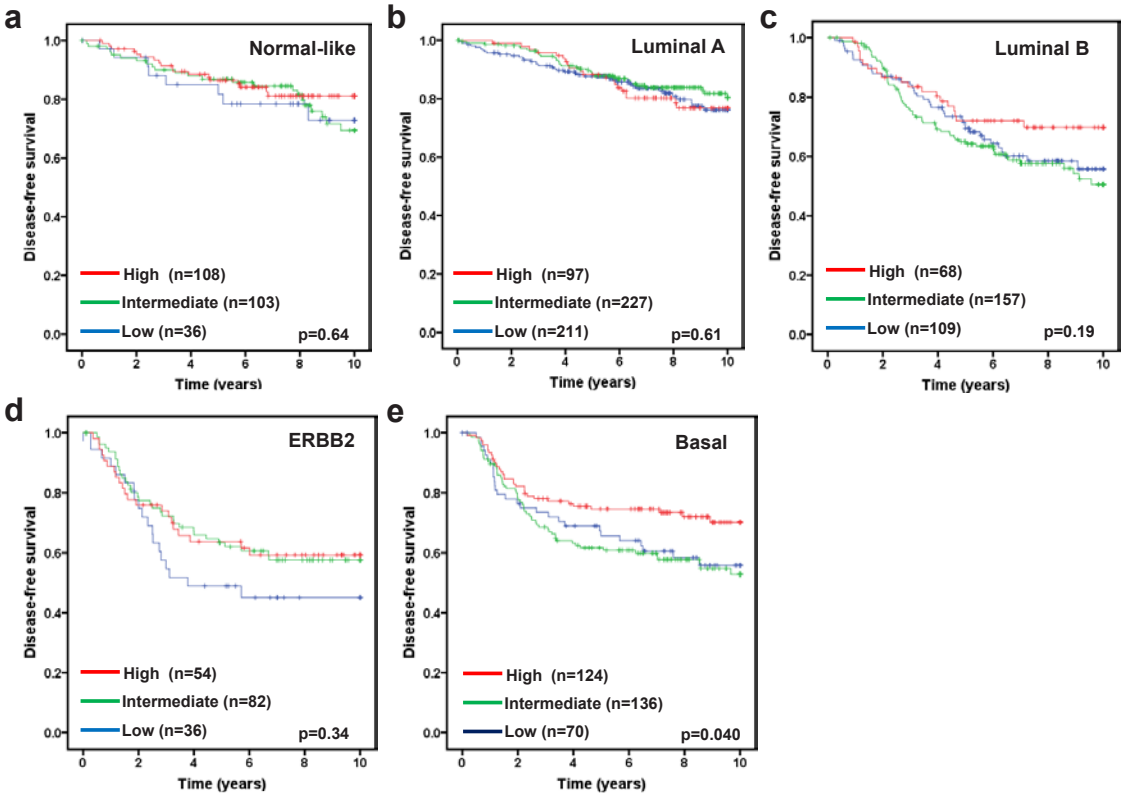

Figure 5: Effect of *FBXW7* expression levels on DFS according to molecular subtypes. Kaplan-Meier estimates of DFS according to the *FBXW7* expression are presented. (a) Nomal-like; (b) Luminal A; (c) Luminal B; (d) ERBB2; and (e) Basal subtype. The p values shown were obtained from a long-rank test among three groups.

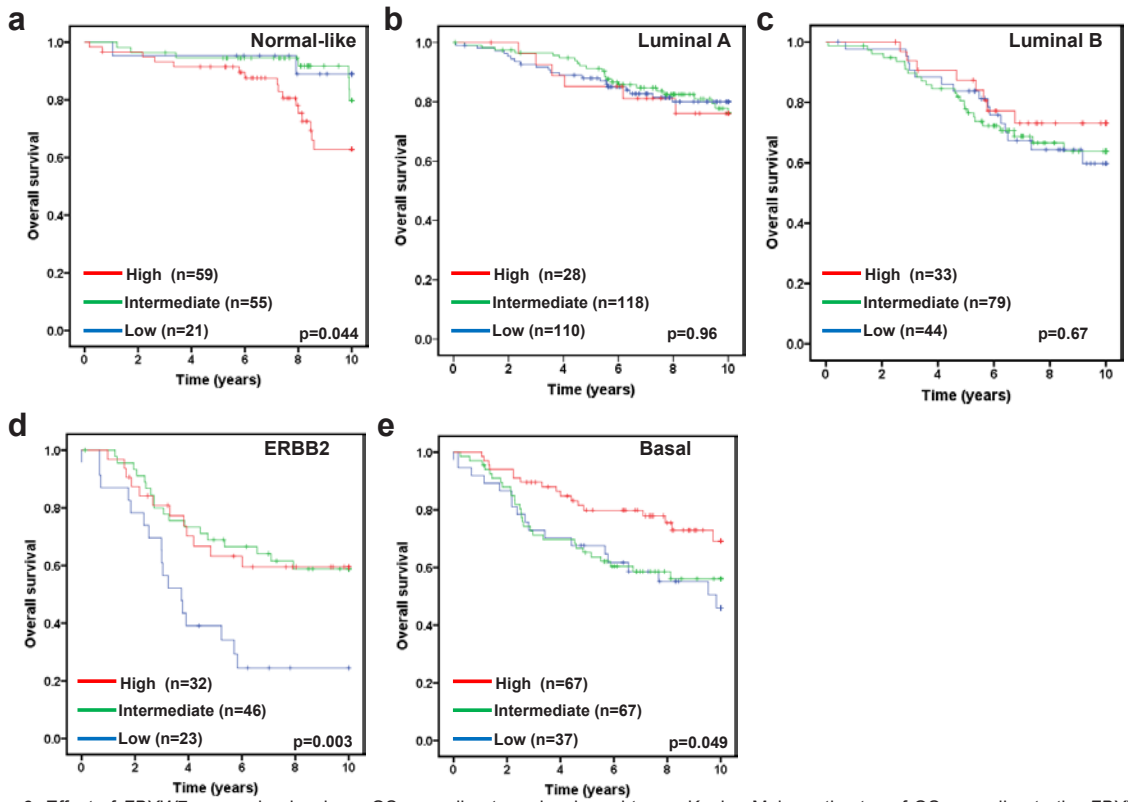

Figure 6: Effect of *FBXW7* expression levels on OS according to molecular subtypes. Kaplan-Meier estimates of OS according to the *FBXW7* expression are presented. (a) Nomal-like; (b) Luminal A; (c) Luminal B; (d) ERBB2; and (e) Basal subtype. The p values shown were obtained from a long-rank test among three groups.

possibly due to that very small proportion of tumors was ER-negative in the study.

On the other hand, there is no effect of *FBXW7* expression on OS in the complete patient sample set. With stratification of patients according to ER status, we found that higher levels of *FBXW7* expression significantly increased OS time in the patients with ER-negative, but not ER-positive tumors. Surprisingly, with stratification of patients according to molecular subtypes, we found the opposite effect of *FBXW7* mRNA levels on OS. In normal-like subtype, the patients in the high *FBXW7* expression group had a significantly poorer OS than those in the low *FBXW7* expression group. In ERBB2 and basal subtype, the patients in the high *FBXW7* expression group had a significantly better OS than those in the low *FBXW7* expression group. The different effects of *FBXW7* on OS in the breast cancer subtypes could reflect the different progression pathways. There is a recent study showing that hypermethylation of *FBXW7β* (reduced expression of *FBXW7β*) is related to favorable OS [38]. The additional studies are needed to get insight into how *FBXW7* functions differently in these different subtypes.

However, our results should be interpreted with caution since there are some limitations of this meta-analysis. Major limitation was unable to explore the potential for confounding by various clinical factors, such as age, disease stage, different treatment regimes. The other limitation is that published articles often lack sufficient information to allow adequate assessment of the quality of the study, which subsequently influences the confident level of meta-analysis. Finally, the studies included in this meta-analysis were from different populations, it is possible that demographic factors can confound our results.

Conclusion

The meta-analysis of transcriptional profiles showed that *FBXW7* expression may be useful as a prognostic factor for patients with ER-negative and basal subtype tumors.

Acknowledgments

We thank Dr. Erik Fredlund (University of California, San Francisco) for providing us the microarray data and clinic information of Dataset 1 to 8. GW is supported by National Natural Science Foundation of China No. 81172528, and Doctoral Fund of Ministry of Education of China No. 20110131110035. JHM is supported by the National Institutes of Health, National Cancer Institute grant R01 CA116481, the Low Dose Scientific Focus Area, Office of Biological & Environmental Research, US Department of Energy (DE-AC02-05CH11231), and Laboratory Directed Research & Development Program (LDRD).

The authors have no conflict of interest.

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