Original Article

Significance of BMI1 and FSCN1 Expression in Colorectal Cancer

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ABSTRACT

Background/Aims: Colorectal cancer (CRC) is the third most common type of cancer in terms of incidence and the fourth in cause of death world-wide, underscoring the need to identify novel biomarkers for early diagnosis, as well as improved disease stratification and treatment choices. **Patients and Methods:** The Gene Expression Omnibus (GSE21510) and the Cancer Genome Atlas (TCGA) CRC datasets were utilized in the current study. GeneSpring 13.0 was used for normalization and analysis. The log-rank test was used to compare the outcome between expression groups. **Result:** Significant upregulation of BMI1 (2.3 FC, $P = 3.7 \times 10^{-18}$) and FSCN1 (1.3 FC, $P = 4.7 \times 10^{-3}$) was observed in CRC. High BMI1 expression was associated with reduced overall survival (OS) [Hazard ratio (HR), 1.87; 95% CI. 1.17–3.03; $P = 0.009$] and reduced disease-free survival (DFS) [HR, 162; 95% CI 1.01–2.63; $P = 0.045$]. Similarly, high expression of FSCN1 was associated with reduced OS (HR, 2.0; 95% CI, 1.24–3.2; $P = 0.0044$) and reduced DFS (HR, 1.60; 95% CI, 0.99–2.57; $P = 0.055$). Importantly, $BMI1^{high}/FSCN1^{high}$ patients experienced the worst OS (HR, 3.17; 95% CI, 1.77–6.15; $P = 0.0002$) and DFS (HR, 2.34; 95% CI, 1.27–4.67, $P = 0.0078$). Using pathway analyses, tumors overexpressing BMI1 were enriched in zinc finger proteins and genes involved in DNA binding and regulation of transcription, whereas tumors expressing FSCN1 were enriched in genes involved in cell migration. **Conclusion:** Our data revealed poor OS and DFS in CRC patients overexpressing BMI1 or FSCN1 and suggest that these two markers in combination may represent superior prognostic marker to either one. Targeting BMI1 and FSCN1 may also provide potential therapeutic opportunity in CRC.

Key Words: BMI1, CRC, FSCN1, survival

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Colorectal cancer (CRC) is among the most prevalent types of cancers causing high mortality rates globally. It is ranked as the third leading cause of death in both genders.[1] In the Kingdom of Saudi Arabia, CRC is the most common cancer type in men and the second most common in women.[2] The 5-year overall survival (OS) has been reported to be ~63% for patients with localized disease, ~50% for patients with regional disease, and ~15% for patients with distant metastases.[2] Several genes have been implicated in CRC development and therapy failure. Polycomb group (PcG) family of genes has recently emerged as key regulators of cancer development and progression, which function by forming several multimeric polycomb repressive complexes, such as PRC1 and PRC2.[3,4] PRC1, also referred to as the "maintenance complex," contains the BMI1 proto-oncogene.[5] In addition to their role during development, different members of the PcG gene family have altered expression in different cancer types. We previously reported a novel role for BMI1 polycomb gene in promoting cancer cell survival through inhibition of the p53-dependant cell death.[6] BMI1 expression has been linked to the cancer stem cell phenotype and its expression was correlated with disease progression and poor clinical outcome in different human malignancies.[7-9] Our recent work revealed multiple dysregulated

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networks in CRC in Saudi Arabia, affecting different cellular processes and pathways (cell cycle, integrated cancer, Wnt, matrix metalloproteinase, and TGF-β).[10] Among the identified genes, we found FSCN1 to be upregulated in CRC compared to adjacent normal tissue (Supplementary Figure 1). FSCN1 is an actin-binding protein which is oftentimes upregulated in different human cancers.[11,12] In particular, overexpression of FSCN1 promoted cancer cell migration, invasion, and metastasis *in vitro* and *in vivo*.[13-15] While various studies have examined the expression of BMI1 or FSCN1 in various human cancers, none to-date has examined the coexpression of BMI1 and FSCN1 in CRC and identified the molecular phenotype of BMI1 and FSCN1 expressing tumors. In the current study, we assessed the expression and clinical significance of BMI1 and FSCN1 in two different CRC cohorts and revealed the molecular phenotype of tumors expressing these two markers.

MATERIALS AND METHODS

Patient information and data analysis

The current study was conducted on two different CRC cohorts. First, the NCBI Gene Expression Omnibus (GEO) dataset (http://www.ncbi.nlm.nih.gov/gds, GSE21510 was utilized to compare the expression of BMI1 and FSCN1 in CRC (n = 123) to normal controls (n = 25). Second, log2 normalized RNAseq expression data were retrieved from a total of 360 colorectal cancer patients from the Cancer Genome Atlas (TCGA) portal (http://www.cbioportal.gov), as described before.[16,17] Patients were subsequently divided into high and low according to the median expression of BMI1 or FSCN1, as we previously described.[4] The clinical characteristics of the TCGA dataset are shown in Table 1. The clinical characteristics for the GSE21510 has been described before.[18] The clinical characteristics for patients from the King Khalid University Hospital dataset were previously described.[10]

Microarray data analysis

Raw gene expression microarray data were retrieved from the GEO (GSE21510) and were imported into GeneSpring 13.0 (Agilent Technologies, Palo Alto, CA, USA). Raw data were subsequently normalized using percentile Shift, whereas Benjamini–Hochberg False Discovery Rate (FDR) method was used for multiple testing corrections. Two-fold cutoff and P (corr) < 0.05 were used to determine significantly changed transcripts.

Statistical analysis

Survival curve comparison was conducted using the log-rank (Mantel–Cox) test. Unpaired two-tailed t-test and P value of <0.05 was considered significant. Pearson correlation was used to identify genes with similar expression pattern to that of BMI1 or FSCN1 in CRC. Mukaka[19] has previously suggested using 0.3 correlation coefficient for Pearson correlation studies, hence a correlation of ≥0.3 cutoff was considered significant. Pathway analyses were conducted using The Database for Annotation, Visualization and Integrated Discovery functional annotation and clustering bioinformatics tool, as we previously described.[4] Statistical analyses and graphing were performed using Graphpad Prism 6.0 software (Graphpad® Software, San Diego, CA, USA).

RESULTS

BMI1 and FSCN1 are overexpressed in colorectal cancer

The expression level of BMI1 and FSCN1 were assessed in a cohort of 123 CRC and 25 normal subjects retrieved from the GEO (GSE21510). Data presented in Figure 1a and b revealed significant upregulation of BMI1 (2.3 FC, $P = 3.7 \times 10^{-18}$) and FSCN1 (1.3 FC, $P = 4.7 \times 10^{-3}$) in CRC, respectively.

Expression of BMI1 and FSCN1 correlates with poor clinical outcome

We subsequently sought to assess the significance of overexpression of BMI1 and FSCN1 in CRC. Therefore, the expression of BMI1 and FSCN1 was interrogated in the TCGA CRC cohort (360 patients). When the patients were divided into two groups according to the median expression, patients with BMI1^{high} exhibited significantly lower OS [56.2 month median survival for BMI1^{high} vs. 99.9 month for BMI1^{low}; HR, 1.87; 95% confidence interval, 1.17–3.03; $P = 0.009$, log-rank (Mantel–Cox) test; Figure 2a]. Similarly, patients with FSCN1^{high} exhibited significantly lower OS [67.3 month median survival for FSCN1^{high}

Table 1: The Cancer Genome Atlas dataset patient and tumor characteristics

	N=360	%
Age, years		
Median age	66	
Range	31-90	
Gender		
Male	201	55.8
Female	159	44.2
Overall survival, months		
Median	15.9	
Range	0-140	
Disease-free survival, months		
Median	15.0	
Range	0-140	
Stage		
I	55	15.3
II	130	36.2
III	112	31.2
IV	50	13.9
NA	12	3.3

vs. 92.7 month for FSCN1^{low}; HR, 2.0; 95% confidence interval, 1.24–3.20; $P = 0.0044$, log-rank (Mantel–Cox) test; Figure 2b]. DFS also followed similar trends where BMI1^{high} patients exhibited significantly lower DFS [37.0 month median survival for BMI1^{high} vs. 109.0 month for BMI1^{low}; HR, 1.62; 95% confidence interval, 1.01–2.63; $P = 0.045$, log-rank (Mantel–Cox) test; Figure 3a]. FSCN1^{high} patients showed significantly lower DFS [74.6 month median survival for FSCN1^{high} vs. 109.0 month for FSCN1^{low}; HR, 1.60; 95% confidence interval, 0.99–2.57; $P = 0.055$, log-rank (Mantel–Cox) test; Figure 3b].

Combination of BMI1 and FSCN1 expression has more predictive value than each marker alone

We subsequently assessed the predictive value of the combination of BMI1 and FSCN in CRC. Interestingly,

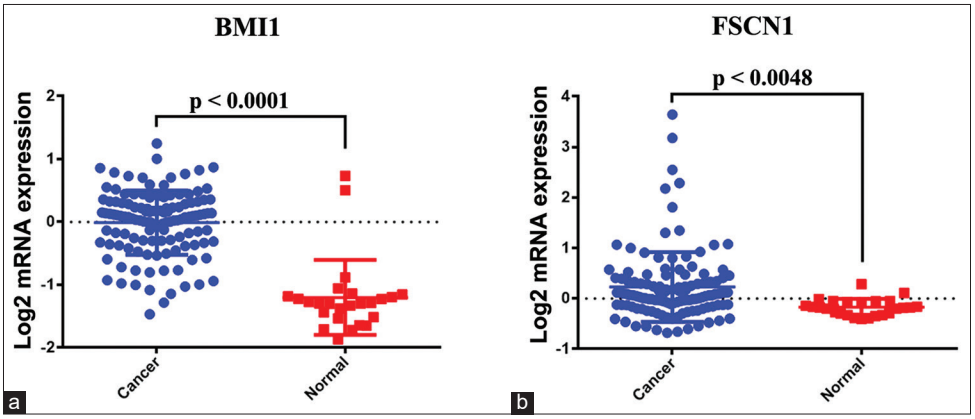

Figure 1: BMI1 and FSCN1 are overexpressed in colorectal cancer. (a) Scatter plot depicting significant upregulation of BMI1 in colorectal cancer patients ($n = 123$) compared to normal tissue ($n = 25$) based on microarray expression. (b) FSCN1 is significantly upregulated in colorectal cancer patients ($n = 123$) compared to normal tissue ($n = 25$) based on microarray expression

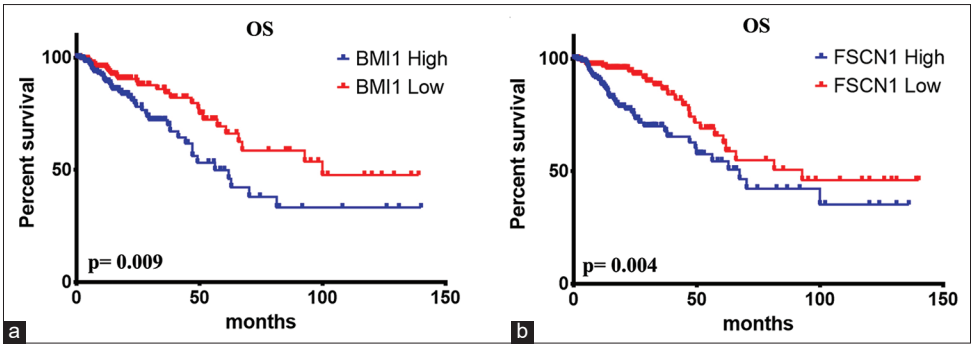

Figure 2: Poor overall survival in colorectal cancer patients overexpressing BMI1 or FSCN1. Kaplan–Meier plot of overall survival in the The Cancer Genome Atlas colorectal cancer cohort as a function of (a) BMI1 or (b) FSCN1 expression, divided according to median expression. Log-rank (Mantel–Cox) test was used to calculate statistical significance

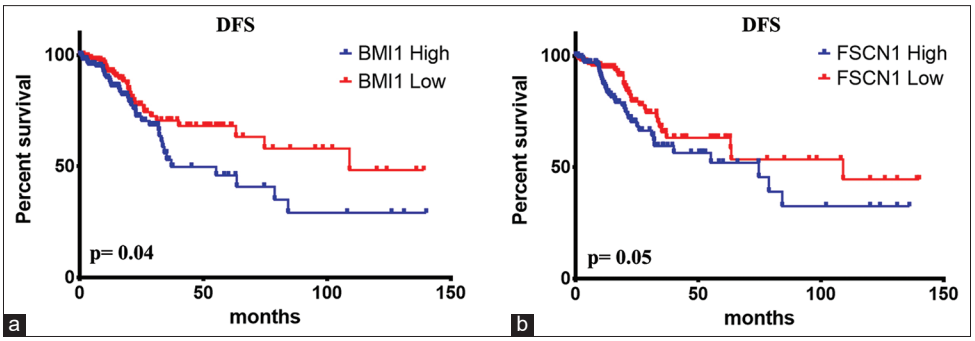

Figure 3: Poor disease free survival in colorectal cancer patients overexpressing BMI1 or FSCN1. Kaplan–Meier plot of disease free survival in the The Cancer Genome Atlas colorectal cancer cohort as a function of (a) BMI1 or (b) FSCN1 expression, divided according to median expression. Log-rank (Mantel–Cox) test was used to calculate statistical significance

BMI1^{high}/FSCN1^{high} tumors were associated with reduced OS [47.0 month median survival for BMI1^{high}/FSCN1^{high} vs. >139.0 month for BMI1^{low}/FSCN1^{low}; HR, 3.17; 95% confidence interval, 1.77–6.15; $P = 0.0002$, log-rank (Mantel–Cox) test; Figure 4a] and reduced DFS [55.1 month median survival for BMI1^{high}/FSCN1^{high} vs. >109.0 month for BMI1^{low}/FSCN1^{low}; HR, 2.34; 95% confidence interval, 1.27–4.67, $P = 0.0078$, log-rank (Mantel–Cox) test; Figure 4b]. Therefore, the combination of BMI1 and FSCN1 demonstrated better prognostic value than the utilization of each marker alone (compare Figure 4 to Figures 2 and 3).

Pathway analysis revealed the molecular phenotype of BMI1 and FSCN1 expressing colorectal cancer tumors

We subsequently sought to determine the molecular phenotype of BMI1 vs. FSCN1 expressing tumors in CRC. Using network analysis, BMI1 and FSCN1 were found to be at the center of two overlapping cellular networks [Figure 5a]. Using Pearson correlation (≥0.3), we identified 425 genes whose expression followed similar pattern to that of BMI1 in CRC [Supplementary Table 1]. Functional annotation and clustering analysis performed on this gene list revealed prominent enrichment in zinc finger and DNA binding protein and those genes involved in the regulation of gene expression. On the other hand, Pearson correlation identified 602 genes whose expression positively correlated with FSCN1 expression in CRC [Supplementary Table 2]. Functional annotation and clustering analysis on the identified gene list revealed prominent enrichment in genes involved in regulating cell migration.

DISCUSSION

CRC is the third most common type of cancer in incidence and the fourth cause of cancer death world-wide, underscoring the need to identify novel biomarkers for early diagnosis, as well as for improved disease stratification and treatment choices. Treatment choice for CRC varies according to tumor location and stage at diagnosis.[20,21] Surgery is the most common treatment for early-stage (stage I and II) CRC. For patients with stage III CRC, surgery is frequently followed by short chemotherapy (approximately 6 months) to decrease the risk of recurrence.[22,23]

Chemotherapy is usually a common treatment for patients with advanced disease. The 5-year relative survival rates for patients with CRC is 64.9%, and when the disease spreads to distant organs, the 5-year survival rate drops to 12.5%.[24] Therefore, there is a need for early diagnosis, better predictive markers, and to offer better treatment modalities for this disease.

Cumulative evidence revealed a functional involvement of different genes and signaling pathways in cancer development and resistance to standard therapies. For instance, the cancer stem cell (CSC) hypothesis proposes that tumors are heterogeneous and only a small fraction of tumor cells (CSC) is tumorigenic and those are the cells that resist standard therapies.[7,25] BMI1 is a member of the PcG, which function by forming several multimeric polycomb repressive complexes, such as PRC1, and PRC2. PRC1, also known as the "maintenance complex," contains the BMI1 proto-oncogene. In stem cells, PcG genes maintain stemness by repressing transcription of key stem cell genes, such as homeobox (Hox) genes, and by preventing senescence through repression of the INK4A locus.[26,27] In addition to their role during development of stem cells, several members of the PcG gene family are overexpressed in different cancer types. In particular, several studies correlated BMI1 overexpression with disease progression and poor clinical outcome.[8,9] We recently characterized a novel role for BMI1 in protecting cancer cells from P53-mediated apoptosis during radiation therapy, which would be concordant with the observed

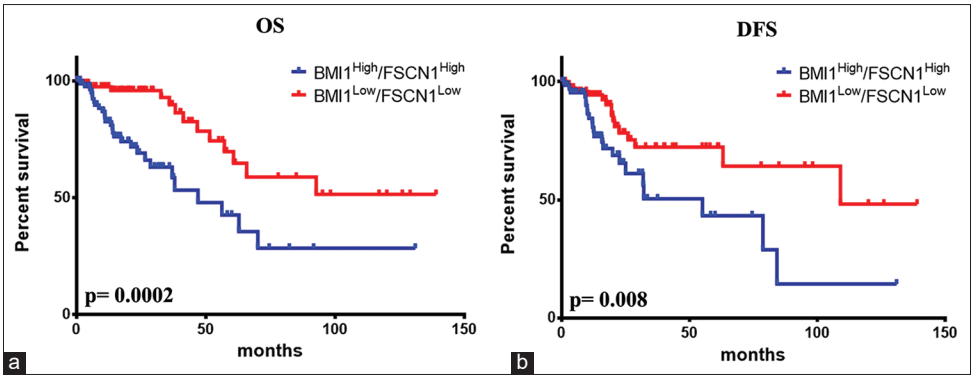

Figure 4: Combination of BMI1 and FSCN1 expression has higher predictive value than either marker alone. Kaplan–Meier plot of (a) overall survival or (b) disease free survival, in the The Cancer Genome Atlas colorectal cancer cohort as a function of BMI1/FSCN1 expression comparing BMI1^{high}/FSCN1^{high} to BMI1^{low}/FSCN1^{low}, divided according to median expression. Log-rank (Mantel–Cox) test was used to calculate statistical significance

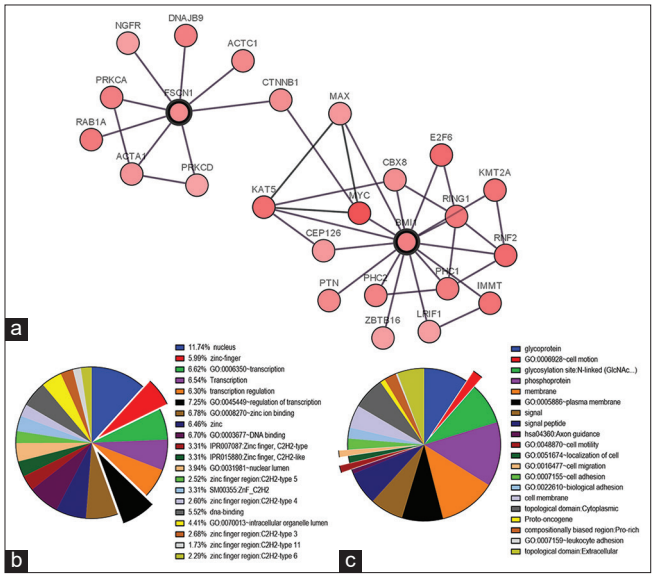

Figure 5: Molecular characterization of BMI1 and FSCN1 expressing colorectal cancer. (a) Network analysis performed on BMI1 and FSCN1 in colorectal cancer revealed two overlapping cellular networks where BMI1 and FSCN1 at the center of those networks. (b) Functional annotation of the gene list identified in BMI1 expressing colorectal cancer tumors revealed enrichment in genes involved in DNA binding and regulation of transcription. Data are presented as pie chart which illustrates the distribution of the top 20 pathway designations in BM1 expressing colorectal cancer tumors. The pie size corresponds to the number of matched entities in each pathway. (c) Functional annotation of the gene list identified in FSCN1 expressing colorectal cancer tumors revealed enrichment in genes involved in cell migration. Data are presented as pie chart which illustrates the distribution of the top 20 pathway designations in FSCN1 expressing colorectal cancer tumors. The pie size corresponds to the number of matched entities in each pathway

poor OS and DFS in the current study.[6] Nonetheless, a number of studies implicated overexpression of BMI1 in the tumor-initiating cells (TI-C) of leukemia, brain, or breast cancers.[28-30] Therefore, it could be postulated that BMI1 is conferring resistance in the subset of TI-Cs during conventional therapy, which would also be concordant with our findings in the current study. Consistent with its role in stem cell maintenance, our data has revealed that BMI1-expressing cells are enriched in zinc finger, DNA binding, and genes involved in transcriptional regulation [Figure 5a and b]. Therefore, it is plausible that the poor OS and DFS observed in BMI1^{high} CRC patients can be attributed to the enrichment in a CSC phenotype.

On the other hand, our recent molecular profiling of CRC in Saudi Arabia has revealed multiple dysregulated pathways in CRC.[10] Interestingly, our data revealed significant upregulation of FSCN1 in CRC, which would be consistent with the data presented in the current study from other datasets (GSE21510). Our current study has revealed enrichment in genes involved in cell migration in FSCN1^{high} CRC tumors, which might explain the observed reduced OS and DFS in those patients [Figures 2 and 3]. Interestingly, tumors expressing high levels of BMI1 and FSCN1 exhibited significant decline in OS and DSF compared to patients expressing either marker alone [Figure 4 vs. Figures 2 and 3]. Therefore, our data suggest that tumors that exhibit a CSC phenotype (BMI1^{high}) and a high migration and metastatic potential (FSCN1^{high}) are the tumors that cause relapse faster than tumors lacking this phenotype.

CONCLUSION

Our data revealed poor OS and DFS in CRC patients overexpressing BMI1 or FSCN1 and suggest using combination of the two markers as a novel prognostic indicator in CRC. Targeting BMI1 and FSCN1 might provide potential therapeutic opportunity for CRC.

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Conflicts of interest

There are no conflicts of interest.

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