SMARCA2 (BRM) expression is associated with enhanced survival in patients with breast cancer.

Shahan Mamoor, MS¹

¹Thomas Jefferson School of Law

San Diego, CA 92101

mamoorsk@tjssl.edu

SMARCA2, also known as Brahma (BRM) (1) is a component of the mammalian switch sucrose non-fermentable (SWI/SNF) chromatin remodeling complex that interacts with nucleosomes, the proteins that bind DNA, to alter the confirmation of the genetic code and thereby regulate transcription (2, 3). Subunits of the SWI/SNF complex are recurrently mutated in a wide variety of cancers (4-6). We mined published tumor transcriptome data with paired survival data to discover genes associated with survival outcomes in breast cancer (7, 8). We found that SMARCA2 was among the genes most differentially expressed in both primary and metastatic tumor tissues when comparing tumor transcriptomes based on survival at 24 months. SMARCA2 expression was significantly higher in the metastatic tumors of patients surviving greater than 24 months, suggesting that increased SMARCA2 expression confers a survival benefit to patients with metastatic breast cancer.

Keywords: breast cancer, metastatic breast cancer, SWI/SNF, SMARCA2, Brahma, BRM, nucleosome remodeling, chromatin dynamics in cancer, targeted therapeutics in breast cancer, tumor transcriptome analysis.

Chromatin remodeling is the process by which the 3-dimensional conformation of DNA is altered to enable or disable transcription of genes, linking chromatin conformation to transcriptional regulation (2). SWI/SNF is a chromatin remodeling complex composed of multiple subunits, including one of two mutually exclusive ATPase enzymatic subunits, one of which is SMARCA2 (1). The gene products of at least eight SWI/SNF genes are found to be mutated in human cancers, and the second ATPase enzymatic subunit of the SWI/SNF complex, a homolog of SMARCA2, SMARAC4 has known tumor suppressive function (9-11).

Patients with metastatic breast cancer possess poor survival outcomes, with a 5 year overall survival rate of 27% (12). To facilitate a therapeutic target and an understanding of the basic transcriptional nature of metastatic breast cancer we mined published microarray data from the primary and metastatic tumors of patients with breast cancer with paired survival outcome data (7, 8). By comparing the transcriptomes of metastatic tumor tissues from patients with breast cancer, we found that the SMARCA2 was among the genes whose expression most significantly differed between those surviving more or less than 24 months. Analysis of a separate dataset revealed that SMARCA2 expression was also associated with disease-free survival at 24 months when comparing the transcriptomes of primary breast tumors. These data indicate that increased SMARCA2 expression is associated with enhanced survival in human breast cancer and suggest tumor suppressive functions for SMARCA2 in breast cancer.

Methods

We used datasets GSE48391 (7) and GSE124647 (8) in conjunction with GEO2R for this comparative differential gene expression analysis of the breast cancer transcriptome.

GSE48391 (7) was generated using Affymetrix Human Genome U133 Plus 2.0 Array technology with $n=70$ for primary tumors of the breast from women with disease-free survival greater than 24 months and an $n=11$ for primary tumors of the breast from women with disease-free survival less than 24 months.

GSE124647 (8) was generated using Affymetrix Human Genome U133 Array from biopsies from patients with stage IV metastatic breast cancer with an $n=70$ for metastatic tumor tissues from women surviving greater than 24 months and an $n=70$ for metastatic tumor tissues from women surviving less than 24 months.

The Benjamin and Hochberg method of p-value adjustment was used for ranking of differential expression but raw p-values were used for assessment of statistical significance of global differential expression. Log-transformation of data was auto-detected, and the NCBI generated category of platform annotation was used.

A statistical test was performed to evaluate the significance of difference between mRNA expression levels of SMARCA2 in the primary tumors of women surviving less than 24 months as compared to the primary tumors of women surviving greater than 24 months, as well as in the metastatic tissues of women surviving less than 24 months as compared to the metastatic tissue of women surviving greater than 24 months using a two-tailed, unpaired t-test with Welch's correction. Only p-values less than 0.05 were considered statistically significant. We used PRISM for all statistical analyses (Version 8.4.0)(455).

Results

We mined published microarray data to perform a comparative transcriptome analysis of tumors from patients with breast cancer and discover genes associated with enhanced survival outcomes.

SMARCA2 is differentially expressed in the primary breast patients with breast cancer that have disease-free survival greater than 24 months when compared to those with disease-free survival less than 24 months.

We identified SMARCA2 as one of the genes most differentially expressed in the tumors of patients when comparing transcriptomes based on disease-free survival at 24 months.

1 When sorting each transcript measured by microarray based on change in expression
2 between tumors from patients with disease-free survival less than 24 months versus tumors
3 from patients with disease-free survival greater than 24 months, a transcript encoded by the
4 SMARCA2 gene ranked 189 out of 54674 transcripts. Differential expression of SMARCA2 in
5 primary breast tumors from patients with disease-free survival greater than 24 months was
6 statistically significant (Table 1; $p=0.00190539$).
7

8
9 SMARCA2 is differentially expressed in the metastatic tumor tissues of patients with breast
10 cancer that have overall survival greater than 24 months when compared to those with overall
11 survival less than 24 months.
12

13 We further analyzed a separate microarray dataset, this containing transcriptome data
14 from the metastatic tissues from multiple organ sites from patients with stage IV metastatic
15 breast cancer. Again, we identified SMARCA2 as among the most differentially expressed
16 genes in metastatic tumor tissues when comparing tumor transcriptomes based on overall
17 survival at 24 months.
18

19 When sorting each transcript measured by microarray based on change in expression
20 between metastatic tumors from patients with overall survival greater than 24 months versus
21 metastatic tumors from patients with disease-free survival less than 24 months, a transcript
22 encoded by the SMARCA2 gene ranked 210 out of 22283 transcripts. Differential expression
23 of SMARCA2 in primary breast tumors from patients with disease-free survival greater than 24
24 months was statistically significant (Table 1; $p=8.96E-04$).
25

26 SMARCA2 is expressed at significantly higher levels in the primary tumors of patients with
27 breast cancer with disease-free survival greater than 24 months as compared to the primary
28 tumors of patients with breast cancer with disease-free survival less than 24 months.

1 We extracted exact mRNA expression levels of SMARCA2 from each patient tumor and
2 performed a statistical test to determine whether the difference in expression between survival
3 groups was statistically significant. SMARCA2 was expressed at significantly higher levels in
4 the primary tumors of breast cancer patients with disease-free survival greater than 24 months
5 as compared to that of patients with disease-free survival less than 24 months, and this
6 difference was statistically significant (Figure 1; $p=0.0151$).

7
8 SMARCA2 is expressed at significantly higher levels in the metastatic tumor tissues of patients
9 with breast cancer with overall survival greater than 24 months as compared to the primary
10 tumors of patients with breast cancer with overall survival less than 24 months.

11
12 We also extracted exact mRNA expression levels of SMARCA2 from each metastatic
13 tumor tissue and performed a statistical test to determine whether the difference in expression
14 between survival groups was statistically significant. SMARCA2 was expressed at significantly
15 higher levels in the metastatic tumor tissues of stage IV metastatic breast cancer patients with
16 overall survival greater than 24 months as compared to that of patients with overall survival
17 less than 24 months, and this difference was statistically significant (Figure 2; $p=0.0011$).

18
19
20 Thus, we identified SMARCA2 as a differentially expressed in human breast cancer,
21 both in primary tumors and in metastatic tumors, when comparing patients based on survival
22 outcomes at 24 months. SMARCA2 was expressed at significantly higher levels in primary
23 tumors from breast cancer patients with disease-free survival greater than 24 months. In
24 patients with stage IV metastatic breast cancer, SMARCA2 was expressed at significantly
25 higher levels in metastatic tumors from patients with overall survival greater than 24 months.

26 27 **Discussion**

28 The SWI/SNF complex is recurrently mutated in a wide variety of human cancers, and
genetic studies using mice have experimentally validated tumor suppressor functions for SWI/

1 SNF subunits including SMARCA4, also known as BAF (barrier to auto-integration factor) (4, 5,
2 6). SMARCA2 is the homolog of SMARCA4; SMARCA2 and SMARCA4 are mutually exclusive
3 components of a SWI/SNF complex (1, 6).

4 The SMARCA2 gene is mutated and its expression is reduced or lost in multiple cancer
5 types (13-19). In human prostate cancers, SMARCA2 protein levels are decreased and
6 SMARCA2 has anti-proliferative effects that antagonize androgen signaling; reduction
7 SMARCA2 of expression in the prostate is associated with an increased proliferative index (13).
8 A study of selected malignant rhabdoid tumors identified trisomy of chromosome 9 in two
9 patients, harboring SMARCA2 (14). A study of lung cancers using tissue microarray revealed
10 that 6.4% of lung adenocarcinomas had lost SMARCA2 expression while 1.7% of squamous
11 cell carcinomas had lost SMARCA2 expression (15). In the hypercalcemic type of ovarian small
12 cell carcinoma, somatic and germline mutations of SMARCA2 are present in nearly all cases
13 (16). Three cases of non-melanoma skin cancer were found to harbor de novo point mutations
14 in SMARCA2, one in squamous cell carcinoma and two in basal cell carcinoma (17). An
15 immunohistochemistry study of a cohort of 685 patients with esophageal adenocarcinoma
16 (EAC) revealed that 9.9% of EAC tumors had lost expression of SMARCA2 (18). A study
17 integrating data from the cancer genome atlas and in vitro experiments revealed that the
18 promoter region of SMARCA2 was hyper-methylated and that this hyper-methylation correlated
19 with decreased expression of SMARCA2. Decreased expression of SMARCA2 in patients with
20 lung cancer was associated with poorer survival outcomes (19). Together, these data
21 collectively point to a tumor suppressive role for SMARCA2 across a variety of tissues, as
22 expression of SMARCA2 is lost in cancers and decreased expression of SMARCA2 in patient
23 tumors is associated with poorer survival.

24 Differential expression of SMARCA2 identified here should be validated at the protein
25 level, and in larger and separate cohorts of breast cancer patients. The effect of enforced
26 SMARCA2 expression on tumor cell proliferation and viability should be evaluated in breast
27 cancer cell lines *in vitro* and the effect of enforced expression of SMARCA2 expression in
28

1 tumor growth, metastasis and survival should be evaluated in xenograft breast cancer models
2 *in vivo*.

3 In this study, we found that SMARCA2 was among the genes whose expression was
4 most quantitatively different when comparing the tumors of patients with localized and
5 metastatic breast cancer based on survival outcomes at 24 months. SMARCA2 was
6 expressed at significantly higher levels in the primary tumors of breast cancer patients with
7 disease-free survival greater than 24 months. SMARCA2 was expressed at significantly higher
8 levels in the metastatic tumor tissues of patients with stage IV metastatic breast cancer with
9 overall survival greater than 24 months. Together our findings indicate that SMARCA2
10 expression associated with enhanced survival in patients with breast cancer, including patients
11 with stage IV metastatic breast cancer. Modulation of SMARCA2 expression may be a
12 rationale therapeutic strategy in breast cancer.
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28

References

1. Jancewicz, I., Siedlecki, J.A., Sarnowski, T.J. and Sarnowska, E., 2019. BRM: the core ATPase subunit of SWI/SNF chromatin-remodelling complex—a tumour suppressor or tumour-promoting factor?. *Epigenetics & chromatin*, 12(1), p.68.
2. Whitehouse, I., Flaus, A., Cairns, B.R., White, M.F., Workman, J.L. and Owen-Hughes, T., 1999. Nucleosome mobilization catalysed by the yeast SWI/SNF complex. *Nature*, 400(6746), pp.784-787.
3. Mashtalir, N., D'Avino, A.R., Michel, B.C., Luo, J., Pan, J., Otto, J.E., Zullo, H.J., McKenzie, Z.M., Kubiak, R.L., Pierre, R.S. and Valencia, A.M., 2018. Modular organization and assembly of SWI/SNF family chromatin remodeling complexes. *Cell*, 175(5), pp. 1272-1288.
4. Roberts, C.W. and Orkin, S.H., 2004. The SWI/SNF complex—chromatin and cancer. *Nature Reviews Cancer*, 4(2), pp.133-142.
5. Wilson, B.G. and Roberts, C.W., 2011. SWI/SNF nucleosome remodellers and cancer. *Nature Reviews Cancer*, 11(7), pp.481-492.
6. Helming, K.C., Wang, X. and Roberts, C.W., 2014. Vulnerabilities of mutant SWI/SNF complexes in cancer. *Cancer cell*, 26(3), pp.309-317.
7. Huang, C.C., Tu, S.H., Lien, H.H., Jeng, J.Y., Huang, C.S., Huang, C.J., Lai, L.C. and Chuang, E.Y., 2013. Concurrent gene signatures for han chinese breast cancers. *PLoS One*, 8(10).
8. Sinn, B.V., Fu, C., Lau, R., Litton, J., Tsai, T.H., Murthy, R., Tam, A., Andreopoulou, E., Gong, Y., Murthy, R. and Gould, R., 2019. SET ER/PR: a robust 18-gene predictor for sensitivity to endocrine therapy for metastatic breast cancer. *NPJ breast cancer*, 5(1), pp. 1-8
9. Bultman, S., Gebuhr, T., Yee, D., La Mantia, C., Nicholson, J., Gilliam, A., Randazzo, F., Metzger, D., Chambon, P., Crabtree, G. and Magnuson, T., 2000. A Brg1 null mutation in the mouse reveals functional differences among mammalian SWI/SNF complexes. *Molecular cell*, 6(6), pp.1287-1295.
10. Bultman, S.J., Herschkowitz, J.I., Godfrey, V., Gebuhr, T.C., Yaniv, M., Perou, C.M. and Magnuson, T., 2008. Characterization of mammary tumors from Brg1 heterozygous mice. *Oncogene*, 27(4), pp.460-468.

11. Glaros, S., Cirrincione, G.M., Palanca, A., Metzger, D. and Reisman, D., 2008. Targeted knockout of BRG1 potentiates lung cancer development. *Cancer research*, 68(10), pp. 3689-3696.
12. ACS Cancer Facts & Figures 2019, <https://www.cancer.net/cancer-types/breast-cancer-metastatic/statistics>.
13. Shen, H., Powers, N., Saini, N., Comstock, C.E., Sharma, A., Weaver, K., Revelo, M.P., Gerald, W., Williams, E., Jessen, W.J. and Aronow, B.J., 2008. The SWI/SNF ATPase Brm is a gatekeeper of proliferative control in prostate cancer. *Cancer research*, 68(24), pp. 10154-10162.
14. Rao, Q., Xia, Q.Y., Wang, Z.Y., Li, L., Shen, Q., Shi, S.S., Wang, X., Liu, B., Wang, Y.F., Shi, Q.L. and Ma, H.H., 2015. Frequent co-inactivation of the SWI/SNF subunits SMARCB1, SMARCA2 and PBRM1 in malignant rhabdoid tumours. *Histopathology*, 67(1), pp.121-129.
15. Herpel, E., Rieker, R.J., Dienemann, H., Muley, T., Meister, M., Hartmann, A., Warth, A. and Agaimy, A., 2017. SMARCA4 and SMARCA2 deficiency in non-small cell lung cancer: immunohistochemical survey of 316 consecutive specimens. *Annals of diagnostic pathology*, 26, pp.47-51.
16. Tseng, J.H., Jelinic, P., Schlappe, B.A., Conlon, N., Olvera, N., Dao, F., Mueller, J.J., Hussein, Y., Soslow, R.A. and Levine, D.A., 2016. Abstract B15: Concomitant loss of SMARCA2 and SMARCA4 expression in small cell carcinoma of the ovary, hypercalcemic type.
17. Moloney, F.J., Lyons, J.G., Bock, V.L., Huang, X.X., Bugeja, M.J. and Halliday, G.M., 2009. Hotspot mutation of Brahma in non-melanoma skin cancer. *Journal of investigative dermatology*, 129(4), pp.1012-1015.
18. Schallenberg, S., Bork, J., Essakly, A., Alakus, H., Buettner, R., Hillmer, A.M., Bruns, C., Schroeder, W., Zander, T., Loeser, H. and Gebauer, F., 2020. Loss of the SWI/SNF-ATPase subunit members SMARCF1 (ARID1A), SMARCA2 (BRM), SMARCA4 (BRG1) and SMARCB1 (INI1) in oesophageal adenocarcinoma. *BMC cancer*, 20(1), p.12.
19. Wu, J., He, K., Zhang, Y., Song, J., Shi, Z., Chen, W. and Shao, Y., 2019. Wu, J., He, K., Zhang, Y., Song, J., Shi, Z., Chen, W. and Shao, Y., 2019. Inactivation of SMARCA2 by promoter hypermethylation drives lung cancer development. *Gene*, 687, pp.193-199.

Rank	ID	p-value	t	B	Gene	Gene name
189	217707_x_at	0.00190539	3.21	-1.60839	SMARCA2	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 2

Table 1: SMARCA2 is differentially expressed in the primary tumors of breast cancer patients with disease-free survival greater than 24 months as compared to that of patients with disease-free survival less than 24 months.

The rank of differential expression globally (out of 54674 total transcripts), the probe ID, the p-value of global differential expression, t, a moderated t-statistic, B, the log-odds of differential expression between the two groups compared, the gene and gene name are listed in this chart.

Rank	ID	p-value	t	B	Gene	Gene name
210	206544_x_at	8.96E-04	3.39316985	-0.74601	SMARCA2	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 2

Table 2: SMARCA2 is differentially expressed in the metastatic tumor tissues of breast cancer patients with overall survival greater than 24 months as compared to that of patients with overall survival less than 24 months.

The rank of differential expression globally (out of 22283 total transcripts), the probe ID, the p-value of global differential expression, t, a moderated t-statistic, B, the log-odds of differential expression between the two groups compared, the gene and gene name are listed in this chart.

Figure 1: SMARCA2 is expressed at significantly higher levels in the primary tumors of breast cancer patients with disease-free survival greater than 24 months as compared to that of patients with disease-free survival less than 24 months.

The mRNA expression level of SMARCA2 in each patient tumor is graphically depicted with mean mRNA expression levels marked and p-value evaluating statistical significance of difference in SMARCA2 expression listed. Patients with disease-free survival (DFS) less than 24 months: $n=11$. Patients with disease-free survival (DFS) greater than 24 months: $n=70$.

Figure 2: SMARCA2 is expressed at significantly higher levels in the metastatic tumor tissues of breast cancer patients with overall survival greater than 24 months as compared to that of patients with overall survival less than 24 months.

The mRNA expression level of SMARCA2 in each patient tumor is graphically depicted with mean mRNA expression levels marked and p-value evaluating statistical significance of difference in SMARCA2 expression listed. Patients with overall survival (OS) less than 24 months: $n=70$. Patients with overall (OS) greater than 24 months: $n=70$.