IGKC, the immunoglobulin kappa constant gene segment, is differentially expressed in brain metastatic breast cancer.

Shahan Mamoor, MS¹

¹shahanmamoor@gmail.com

East Islip, NY USA

Metastasis to the brain is a clinical problem in patients with breast cancer¹⁻³. We mined published microarray data⁴⁻⁶ to compare primary and metastatic tumor transcriptomes to discover genes associated with brain metastasis in patients with metastatic breast cancer. We found that the immunoglobulin light chain kappa constant locus encoded by IGKC was among the genes whose expression was most different in the brain metastases of patients with metastatic breast cancer as compared to primary tumors of the breast. We observed a significant correlation between IGKC expression in primary tumors of the breast and distant metastasis-free survival, as well as overall survival. Down-regulation of IGKC may be an important event for metastasis of primary tumor-derived cancer cells to the brain in humans with metastatic breast cancer.

Keywords: breast cancer, metastasis, brain metastases, central nervous system metastases, IGKC, immunoglobulin kappa constant locus, systems biology of breast cancer, targeted therapeutics in breast cancer.

One report described a 34% incidence of central nervous system metastases in trastuzumab-treated patients with breast cancer². This alarmingly high frequency of CNS metastasis events demands an enhanced understanding of the transcriptional makeup of brain metastatic tissues to support identification of therapeutic targets, whether they are treatment related or not. We performed a global comparative analysis of primary and metastatic tumors in patients with brain metastatic breast cancer^{4,5}. We discovered significant differential and decreased expression of IGKC in brain metastatic tissues of patients with metastatic breast cancer.

Methods

We used datasets GSE125989⁴, GSE52604⁵ and GSE43837⁶ for this global differential gene expression analysis of brain metastatic breast cancer in conjunction with GEO2R. GSE125989 was generated using Affymetrix Human Genome U133A 2.0 Array technology with $n=16$ primary tumors from patients with breast cancer and $n=16$ brain metastases from patients with brain metastatic breast cancer. GSE52604 was generated using Whole Human Genome Microarray 4x44K G4112F technology with $n=10$ normal breast tissues and $n=35$ brain metastases from patients with breast cancer, analysis performed using platform GPL6480. GSE43837 was generated using Affymetrix Human X3P Array technology with $n=19$ primary breast tumors and $n=19$ brain metastases from patients with breast cancer, analysis performed using platform GPL1352. The Benjamini and Hochberg method of p -value adjustment was used for ranking of differential expression but raw p -values were used to assess 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 whether IGKC gene expression was significantly between primary breast tumors and brain metastases in humans with breast cancer using a two-tailed, unpaired t-test with Welch's correction. We used PRISM for all statistical analyses of differential gene expression in human breast cancer (Version 8.4.0)(455). For Kaplan-Meier survival analysis, we used the Kaplan-Meier plotter online tool⁷ for correlation of IGKC mRNA expression levels with distant metastasis-free survival (DMFS) in $n=1746$ breast cancer patients, and overall survival in $n=1402$ breast patients.

Results

We performed global comparative transcriptome analysis of the primary and metastatic tumor tissues of patients with brain metastatic breast cancer using published microarray data⁴⁻⁶ to describe the transcriptional landscape of brain metastasis in human breast cancer in an unbiased fashion and for the discovery of novel therapeutic targets.

IGKC is differentially expressed in the brain metastases of patients with brain metastatic breast cancer.

We identified the immunoglobulin light chain kappa constant gene segment, IGKC, as among the genes whose expression was most significantly different in the brain metastases of patients with brain metastatic breast cancer⁴ (Table 1). When sorting each of the genes expressed in brain metastases based on significance of difference as compared to the primary tumors of patients with brain metastatic breast cancer, IGKC ranked 57 out of 22297 total transcripts (Table 1), equating to 99.7% differential expression. Differential expression of IGKC in the brain metastases of patients with brain metastatic breast cancer was statistically significant (Table 1; $p=1.03E-04$).

Cross-validation of differentially expressed genes across datasets in cancer can be difficult and more challenging than when cross-validating across organs and sorted cell populations due to higher levels of heterogeneity in tumors, and differing methods of tumor sampling between laboratories. Thus, we asked whether we could validate differential expression of IGKC when comparing brain metastases from patients with breast cancer to normal breast tissue as opposed to primary tumors of the breast. Comparison of global gene expression profiles of 10 normal breast tissues to 35 brain metastases⁵ revealed that IGKC was among the genes whose expression was most significantly different transcriptome-wide between the breast and brain metastases in patients with breast cancer (Table 2). When sorting each of the genes expressed in brain metastases based on significance of difference in expression between brain metastases and normal breast tissues, IGKC ranked 2360 out of 41093 total transcripts (Table 2), equating to 94.3% differential expression. Differential expression of IGKC in brain metastases from patients with brain metastatic breast cancer in this dataset was statistically significant (Table 2; $p=8.26E-06$).

Despite technical challenges associated with performing differential gene expression analyses across tumor type and tissue type, we found in a third dataset differential expression of IGKC in metastatic brain tumor tissue when compared to primary tumors of the breast in patients with breast cancer (Table 3). When sorting each of the genes expressed in brain metastases based on significance of change in expression as compared to primary tumors of the breast, IGKC ranked 95 out of 61359 total transcripts (Table 3), equating to 99.8% differential expression. Differential expression of IGKC was statistically significant (Table 3; $p=0.00037439$).

IGKC is expressed at significantly lower levels in the brain metastases of patients with brain metastatic breast cancer.

We obtained exact mRNA expression levels for IGKC, in primary tumors of the breast and in brain metastasis of patients with brain metastatic breast cancer to determine magnitude and direction of change in IGKC expression in brain metastatic tissues. IGKC was expressed at lower levels in the brain metastases of patients with breast cancer as compared to primary tumors of the breast, and this difference was statistically significant (Figure 1; $p<0.0001$). We calculated a mean fold change of 0.75 ± 0.18 in IGKC expression when comparing primary tumors of the breast to brain metastatic tissues in patients with breast cancer (Table 1).

We also obtained exact mRNA expression levels for IGKC, in primary tumors of the breast and in brain metastasis of patients with brain metastatic breast cancer from a second dataset to determine magnitude and direction of change in IGKC expression in brain metastatic tissues. IGKC was expressed at higher levels in the brain metastases of patients with breast cancer as compared to primary tumors of the breast, and this difference was statistically significant (Figure 2; $p=0.0280$). We calculated a mean fold change of 0.05 ± 0.14 in IGKC expression when comparing primary tumors of the breast to brain metastatic tissues in patients with breast cancer (Table 2).

IGKC expression associates with survival outcomes in breast cancer.

We performed Kaplan-Meier survival analysis to evaluate correlation between IGKC mRNA expression levels with survival outcomes in patients with breast cancer. In 1746 breast cancer patients, we found a statistically significant correlation between IGKC expression and distant metastasis-free survival (DMFS) (Figure 3; log rank p -value: 0.012 for distant metastasis-free survival, hazard ratio: 0.78 (0.65 - 0.95) (Fig. 3)). Lower IGKC mRNA levels in primary tumors of the breast, like which we observed in metastases to the brain, were a negative prognostic indicator in patients with breast cancer. Median DMFS was 77.23 months for patients with low tumor expression of IGKC while median DMFS was 133.68 months for patients with high tumor expression of IGKC (Table 4).

We also performed Kaplan-Meier survival analysis to evaluate correlation between IGKC mRNA expression levels with overall survival outcomes in patients with breast cancer. In 1402 breast cancer patients, we found a statistically significant correlation between IGKC expression and overall survival (OS) (Figure 4; log rank *p*-value: 0.01 for overall survival, hazard ratio: 0.76 (0.61 - 0.94) (Fig. 4)). Lower IGKC mRNA levels in primary tumors of the breast, like which we observed in metastases to the brain, were a negative prognostic indicator in patients with breast cancer with respect to overall survival. Median OS was 79.2 months for patients with low tumor expression of IGKC while median OS was 123.6 months for patients with high tumor expression of IGKC (Table 5).

Thus, by mining published microarray data⁴⁻⁶ in an unbiased and systematic fashion, we identified IGKC as among the genes whose expression was most different in the brain metastases of patients with breast cancer both when compared to primary tumors of the breast and to the normal breast; IGKC was expressed at significantly lower levels in brain metastases as compared to primary tumors of the breast. IGKC expression in primary tumors of the breast in a large cohort of patients demonstrated a significant correlation with both distant metastasis-free survival and overall survival, with higher expression of IGKC correlating with improved survival in humans with breast cancer.

Discussion

IGKC encodes the immunoglobulin kappa constant locus of the immunoglobulin light chain. Immunoglobulin gene expression is thought to be generally restricted to cells of lymphocytic lineage (i.e., B and T-lymphocytes), but expression of immunoglobulin genes in human breast cancer has previously been described⁸. IGKC was found to be representative of a B-cell gene expression signature in breast cancer, where expression of IGKC at the protein level was observed in a study of 330 paraffin-embedded breast cancer tissues, and IGKC was described as a prognostic indicator, with high expression of IGKC associated with complete response to anthracycline chemotherapy⁹. However, expression of IGKC was localized to the stroma and ascribed to tumor-infiltrating lymphocytes and not intrinsic to the tumor¹⁰. In a separate study of tumor tissues from untreated, node-negative patients, high IGKC expression was associated with superior overall and disease-free survival as compared to patients whose tumors expressed low levels of IGKC, in luminal B carcinomas and in ER-negative cancers but not in luminal A carcinomas¹¹.

We provided evidence here that IGKC is among the genes whose expression is most different in the brain metastases of patients with brain metastatic brain cancer and that low expression of IGKC, like that found in brain metastatic tissues, is significantly correlated with worse distant-metastasis free survival and overall survival. Decreased expression of IGKC may be a reflection of varying degrees of lymphocyte contamination in tissues of the breast as opposed to tissues of the brain, but this appears less likely considering we found this phenomena across three separate datasets. Measuring immunoglobulin chain gene expression or gene rearrangement events in real-time, in the primary and metastatic tumors of patients with breast cancer may provide insights into molecular mechanisms underlying metastasis to the brain in patients with brain metastatic breast cancer, and why we recurrently observe expression of an immunoglobulin gene segment in human breast cancers, cells of non-immune origin. Whether breast cancer cells express or differentially express genes involved in the rearrangement and expression of immunoglobulin genes: recombination activating genes RAG1/2¹²⁻¹⁴, the terminal deoxynucleotidyl transferase TdT^{15,16}, vpreB¹⁷, $\lambda 5$ ¹⁷, activation-induced cytidine deaminase AID¹⁸, and transcription factors early B-cell factor Oct-2¹⁹, EBF²⁰, Pax-5²¹⁻²³, GATA-2²⁴ and Ikaros²⁵, and why these genetic events are triggered in non-lymphocytic cells, is an important question which we are now investigating.

References

1. Lin, N.U., Amiri-Kordestani, L., Palmieri, D., Liewehr, D.J. and Steeg, P.S., 2013. CNS metastases in breast cancer: old challenge, new frontiers.
2. Bendell, J.C., Domchek, S.M., Burstein, H.J., Harris, L., Younger, J., Kuter, I., Bunnell, C., Rue, M., Gelman, R. and Winer, E., 2003. Central nervous system metastases in women who receive trastuzumab-based therapy for metastatic breast carcinoma. *Cancer*, 97(12), pp.2972-2977.
3. Tsukada, Y., Fouad, A., Pickren, J.W. and Lane, W.W., 1983. Central nervous system metastasis from breast carcinoma autopsy study. *Cancer*, 52(12), pp.2349-2354.
4. Iwamoto, T., Niikura, N., Ogiya, R., Yasojima, H., Watanabe, K.I., Kanbayashi, C., Tsuneizumi, M., Matsui, A., Fujisawa, T., Iwasa, T. and Shien, T., 2019. Distinct gene expression profiles between primary breast cancers and brain metastases from pair-matched samples. *Scientific reports*, 9(1), pp. 1-8.
5. Salhia, B., Kiefer, J., Ross, J.T., Metapally, R., Martinez, R.A., Johnson, K.N., DiPerna, D.M., Paquette, K.M., Jung, S., Nasser, S. and Wallstrom, G., 2014. Integrated genomic and epigenomic analysis of breast cancer brain metastasis. *PloS one*, 9(1), p.e85448.
6. McMullin, R.P., Wittner, B.S., Yang, C., Denton-Schneider, B.R., Hicks, D., Singavarapu, R., Moulis, S., Lee, J., Akbari, M.R., Narod, S.A. and Aldape, K.D., 2014. A BRCA1 deficient-like signature is enriched in breast cancer brain metastases and predicts DNA damage-induced poly (ADP-ribose) polymerase inhibitor sensitivity. *Breast Cancer Research*, 16(2), pp.1-10.
7. Györfy, B., Lanczky, A., Eklund, A.C., Denkert, C., Budczies, J., Li, Q. and Szallasi, Z., 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*, 123(3), pp.725-731.
8. Chen, Z., Qiu, X. and Gu, J., 2009. Immunoglobulin expression in non-lymphoid lineage and neoplastic cells. *The American journal of pathology*, 174(4), pp.1139-1148.
9. Schmidt, M., Hellwig, B., Hammad, S., Othman, A., Lohr, M., Chen, Z., Boehm, D., Gebhard, S., Petry, I., Lebrecht, A. and Cadenas, C., 2012. A comprehensive analysis of human gene expression profiles identifies stromal immunoglobulin κ C as a compatible prognostic marker in human solid tumors. *Clinical Cancer Research*, 18(9), pp.2695-2703.
10. Pandey, J.P., Kistner-Griffin, E., Black, L., Namboodiri, A.M., Iwasaki, M., Kasuga, Y., Hamada, G.S. and Tsugane, S., 2014. IGKC and FcγR genotypes and humoral immunity to HER2 in breast cancer. *Immunobiology*, 219(2), pp.113-117.
11. Chen, Z., Gerhold-Ay, A., Gebhard, S., Boehm, D., Solbach, C., Lebrecht, A., Battista, M., Sicking, I., Cotarelo, C., Cadenas, C. and Marchan, R., 2012. Immunoglobulin kappa C predicts overall survival in node-negative breast cancer. *PLoS One*, 7(9), p.e44741.
12. Schatz, D.G., Oettinger, M.A. and Baltimore, D., 1989. The V (D) J recombination activating gene, Rag-1. *Cell*, 59(6), pp.1035-1048.
13. Oettinger, M.A., Schatz, D.G., Gorka, C. and Baltimore, D., 1990. RAG-1 and RAG-2, adjacent genes that synergistically activate V (D) J recombination. *Science*, 248(4962), pp.1517-1523.

14. Chun, J.J., Schatz, D.G., Oettinger, M.A., Jaenisch, R. and Baltimore, D., 1991. The recombination activating gene-1 (RAG-1) transcript is present in the murine central nervous system. *Cell*, 64(1), pp. 189-200.
15. Sale, J.E. and Neuberger, M.S., 1998. TdT-accessible breaks are scattered over the immunoglobulin V domain in a constitutively hypermutating B cell line. *Immunity*, 9(6), pp.859-869.
16. Foa, Robert., Giulia Casorati, Maria Cristina Giubellino, Giuseppe Basso, Raffaella Schirò, Giovanni Pizzolo, Francesco Lauria, M. P. Lefranc, Terence H. Rabbitts, and Nicola Migone. "Rearrangements of immunoglobulin and T cell receptor beta and gamma genes are associated with terminal deoxynucleotidyl transferase expression in acute myeloid leukemia." *The Journal of experimental medicine* 165, no. 3 (1987): 879-890.
17. Karasuyama, H., Kudo, A. and Melchers, F., 1990. The proteins encoded by the VpreB and lambda 5 pre-B cell-specific genes can associate with each other and with mu heavy chain. *The Journal of experimental medicine*, 172(3), pp.969-972.
18. Muramatsu, M., Kinoshita, K., Fagarasan, S., Yamada, S., Shinkai, Y. and Honjo, T., 2000. Class switch recombination and hypermutation require activation-induced cytidine deaminase (AID), a potential RNA editing enzyme. *Cell*, 102(5), pp.553-563
19. Corcoran, L.M., Karvelas, M., Nossal, G.J., Ye, Z.S., Jacks, T. and Baltimore, D., 1993. Oct-2, although not required for early B-cell development, is critical for later B-cell maturation and for postnatal survival. *Genes & development*, 7(4), pp.570-582.
20. Hagman, J., Belanger, C., Travis, A., Turck, C.W. and Grosschedl, R., 1993. Cloning and functional characterization of early B-cell factor, a regulator of lymphocyte-specific gene expression. *Genes & development*, 7(5), pp.760-773.
21. Adams, B., Dörfler, P., Aguzzi, A., Kozmik, Z., Urbanek, P., Maurer-Fogy, I. and Busslinger, M., 1992. Pax-5 encodes the transcription factor BSAP and is expressed in B lymphocytes, the developing CNS, and adult testis. *Genes & development*, 6(9), pp.1589-1607.
22. Schwarz, M., Alvarez-Bolado, G., Urbánek, P., Busslinger, M. and Gruss, P., 1997. Conserved biological function between Pax-2 and Pax-5 in midbrain and cerebellum development: evidence from targeted mutations. *Proceedings of the National Academy of Sciences*, 94(26), pp.14518-14523.
23. Leblanc, N., Harquail, J., Crapoulet, N., Ouellette, R.J. and Robichaud, G.A., 2018. Pax-5 inhibits breast cancer proliferation through MiR-215 up-regulation. *Anticancer research*, 38(9), pp.5013-5026.
24. Nardelli, J., Thiesson, D., Fujiwara, Y., Tsai, F.Y. and Orkin, S.H., 1999. Expression and genetic interaction of transcription factors GATA-2 and GATA-3 during development of the mouse central nervous system. *Developmental biology*, 210(2), pp.305-321.
25. Georgopoulos, K., Bigby, M., Wang, J.H., Molnar, A., Wu, P., Winandy, S. and Sharpe, A., 1994. The Ikaros gene is required for the development of all lymphoid lineages. *Cell*, 79(1), pp.143-156.

Rank	ID	p-value	t	B	FC	Gene	Gene name
57	221651_x_at	1.03E-04	-4.3864897	1.2665	0.75 ± 0.18	IGK///IGKC	immunoglobulin kappa locus/// immunoglobulin kappa constant

Table 1: IGKC is differentially expressed in brain metastases in brain metastatic breast cancer.

Rank of differential expression, probe ID, *p*-value with respect to differential expression, *t*, a moderated *t*-statistic, *B*, the log-odds of differential expression between the two groups compared, fold change (FC) of IGKC expression between the brain metastases of patients with breast cancer and primary tumors of the breast, gene and gene name are listed in this chart.

Rank	ID	p-value	t	B	Gene	Gene name
2360	A_24_P272146	8.26E-06	-5.0060288	3.348776	IGKC	immunoglobulin kappa constant

Table 2: IGKC is differentially expressed in brain metastases in brain metastatic breast cancer.

Rank of differential expression, probe ID, *p*-value with respect to differential expression, t, a moderated t-statistic, B, the log-odds of differential expression between the two groups compared, gene and gene name are listed in this chart.

Rank	ID	p-value	t	B	FC	Gene	Gene name
95	g184847_3p_a_at	0.00037439	-3.8623958	-0.05047	0.05 ± 0.14	IGK// IGKC	immunoglobulin kappa locus// immunoglobulin kappa constant

Table 3: IGKC is differentially expressed in brain metastases in brain metastatic breast cancer.

Rank of differential expression, probe ID, *p*-value with respect to differential expression, *t*, a moderated *t*-statistic, *B*, the log-odds of differential expression between the two groups compared, fold change (FC) of IGKC expression between the brain metastases of patients with breast cancer and primary tumors of the breast, gene and gene name are listed in this chart.

Figure 1: IGKC is expressed at significantly lower levels in the brain metastases of patients with brain metastatic breast cancer.

The mRNA expression level of IGKC in primary tumors of the breast (left) and in the brain metastases of brain metastatic breast cancer patients (right) is graphically represented here with mean IGKC levels marked and the result of a statistical test evaluating difference in mRNA expression level between brain metastases and primary tumors of the breast, a *p*-value, listed above.

Figure 2: IGKC is expressed at significantly lower levels in the brain metastases of patients with brain metastatic breast cancer.

The mRNA expression level of IGKC in primary tumors of the breast (left) and in the brain metastases of brain metastatic breast cancer patients (right) is graphically represented here with mean IGKC levels marked and the result of a statistical test evaluating difference in mRNA expression level between brain metastases and primary tumors of the breast, a p -value, listed above. The scale of the Y-axis has been set to natural logarithm.

Figure 3: IGKC expression correlates with distant metastasis-free survival in patients with breast cancer.

Depicted in this Kaplan-Meier plot is the probability of distant metastasis-free survival (DMFS) for $n=1746$ total patients stratified into two groups, based on low or high expression of IGKC in patient primary tumors. The log rank p -value denoting statistical significance of difference in distant metastasis-free survival when comparing the two groups, as well as hazard ratio for this comparison is listed above. Listed below is the number of patients at risk (number of patients alive) per interval, after stratification based on IGKC expression; in the first interval, number at risk is number of patients alive; in each subsequent interval, number at risk is the number at risk less those who have expired or are censored.

Low expression cohort (months)	High expression cohort (months)
77.23	133.68

Table 4: Median distant metastasis-free survival is superior in breast cancer patients with high primary tumor expression of IGKC.

The distant metastasis-free survival of $n=1746$ breast cancer patients based on stratification into low or high tumor expression of IGKC is listed in this chart.

Figure 4: IGKC expression correlates with overall survival in patients with breast cancer.

Depicted in this Kaplan-Meier plot is the probability of overall survival (OS) for $n=1402$ total patients stratified into two groups, based on low or high expression of IGKC in patient primary tumors. The log rank p -value denoting statistical significance of difference in overall survival when comparing the two groups, as well as hazard ratio for this comparison is listed above. Listed below is the number of patients at risk (number of patients alive) per interval, after stratification based on IGKC expression; in the first interval, number at risk is number of patients alive; in each subsequent interval, number at risk is the number at risk less those who have expired or are censored.

Low expression cohort (months)	High expression cohort (months)
79.2	123.6

Table 5: Median overall survival is superior in breast cancer patients with high primary tumor expression of IGKC.

Overall survival of $n=1402$ breast cancer patients based on stratification into low or high tumor expression of IGKC is listed in this chart.