Infection of the kidneys with the BK polyomavirus is associated with transcriptional induction of PSMB9 and IGFLR1, genes differentially expressed in human breast cancer.

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The BK polyomavirus is both the cause of nephropathy in kidney transplant patients¹⁻³ and one of many viruses whose presence has previously been investigated in the tumors of patients with breast cancer^{4,5}. We mined published microarray data^{6,7} to identify differentially expressed genes associated with BK polyomavirus infection of the kidneys in patients with transplant-associated nephropathy, integrating these findings with whole transcriptome analysis of human breast cancers to determine the presence of differentially expressed genes in breast cancers whose expression was significantly modulated by BK virus infection. We identified conserved transcriptional modulation of the IGF-like family receptor 1 (IGFLR1) and the proteasome subunit 9 (PSMB9) in the kidneys of patients with transplant-associated nephropathy and in the primary tumors of patients with breast cancer. These data demonstrate the existence of common transcriptional modulation in human breast cancer and in the kidneys associated with BK virus, and provide rationale for evaluation of BK virus large T-antigen expression in the human breast during breast cancer.

Keywords: breast cancer, breast cancer etiology, BK virus, polyomavirus, BK polyomavirus, BK virus nephropathy, transformation, PSMB9, IGFLR1.

It is estimated that only 5-10%^{10,11} of human breast cancers are inherited (familial), suggesting an unknown etiology in up to 90-95% cases of human breast cancers which totaled over a quarter of a million in the United States in 2019¹². We recently reported that the transcriptional landscape of human breast cancers is strikingly similar to that of murine breast cancers induced by genetic modeling of integration events and oncogene expression during viral infection, like that of the SV40 large T-antigen^{13, 14}. The BK polyomavirus is one of a multitude of genes whose expression has been evaluated in human breast cancers; two studies, using limited methodologies (i.e., not using next-generation sequencing) argued that breast cancer tissues evaluated were negative for the presence of BK viral DNA^{4,5}. However, one study has argued that BK virus may be associated with the development of neuroblastoma¹⁵. One major difficulty in evaluating a role for virally-induced transformation in human cancers^{16,17} is that viral integration and loss of capsid protein expression can result in expression of a viral oncoprotein like the T antigen without detectable productive infection; moreover, transcriptional analyses will be unable to detect the influence of a viral oncoprotein like T antigen which exerts its affect on p53 and/or pRb after transcription and translation of these tumor suppressors, as opposed to somatic mutation which results in the production of altered tumor suppressor DNA and/or RNA. Nevertheless, to more thoroughly assess for any commonalities in gene expression profiles between human breast cancer and polyomaviruses with oncogenic potential, we adopted a blind systems-level approach, mining published microarray data to identify genes most differentially expressed in the kidneys of patients with BK virus nephropathy, as well in the tumors of patients with breast cancer, to identify conserved differentially expressed genes. We identified two genes whose expression was most significantly altered in the kidneys of patients with BK virus nephropathy as significantly differentially expressed in human breast cancer. While the data here are limited in scope, they provide a basis for further interrogation of the presence of BK virus T-antigen expression, or the expression of other viral proteins in the primary tumors of patients with breast cancer.

Methods

We utilized microarray datasets GSE75693⁶, GSE47199⁷, GSE124648⁸, and GSE109169⁹, GSE23938⁹ and for this integrated differential gene expression analysis of BK virus infection of the kidneys in transplant nephropathy, and in female breast cancer in conjunction with GEO2R. GSE75693 was generated using Affymetrix Human Genome U133 Plus 2.0 Array technology with $n=30$ stable kidney graft tissues and $n=15$ BK virus nephropathy biopsy, analysis performed using platform GPL570. GSE47199 was generated using Affymetrix Human Gene 1.0 ST Array technology with $n=11$ normal transplant kidney biopsy and $n=3$ BK virus nephropathy kidney biopsy, analysis performed using platform GPL6244. GSE124648 was generated using Affymetrix Human Genome U133A Array technology with $n=10$ normal breast tissue and $n=126$ tumors of the breast and; analysis was performed using platform GPL96. GSE109169 was generated using Affymetrix Human Exon 1.0 ST Array technology with $n=25$ normal breast tissue and $n=25$ tumors of the breast and; analysis was performed using platform GPL5175. The tissues from this dataset are paired tissues (25 tumors matching 25 breast tissues from 25 patients). 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 target gene expression was significantly between non-

1 affected kidney tissue and BK virus nephropathy-affected kidney tissue using a two-tailed,
2 unpaired t-test with Welch's correction. We used PRISM for all statistical analyses of differential
3 gene expression (Version 8.4.0)(455).

4 **Results**

5 We integrated data from four microarray datasets⁶⁻⁹ to identify, in an unbiased fashion
6 and at the systems-level, conserved differentially expressed expression targets in the kidneys
7 of transplant patients with BK virus nephropathy and in the primary tumors of patients with
breast cancer.

8 **The proteasomal subunit beta 9 is differentially expressed in BK virus nephropathy** 9 **in kidney transplant patients.**

10 We identified PSMB9 as among the genes whose expression was most significantly
11 different when comparing stable kidney graft tissues from transplant patients to that of patients
12 with BK virus-positive transplant nephropathy (BKVN). When sorting each of the transcripts
13 measured in the kidneys of patients with BKVN as compared to that of patients with stable
kidney graft, PSMB9 ranked 50 out of 54675 total transcripts (Table 1). Differential expression
of PSMB9 in BK virus nephropathy was statistically significant (Table 1; $p=1.71E-07$).

14 We queried a second microarray dataset to determine if we could independently
15 validate PSMB9 differential expression in BK virus nephropathy. We again identified PSMB9 as
16 among the genes whose expression was most significantly different when comparing normal
17 kidney transplant biopsy to biopsy tissue from patients with BK virus nephropathy (BKVN).
18 When sorting each of the transcripts measured in the kidneys of patients with BKVN as
19 compared to that of patients with normal kidney transplant, PSMB9 ranked 145 out of 29096
20 total transcripts (Table 2). Differential expression of PSMB9 in BK virus nephropathy was again
statistically significant (Table 2; $p=1.29E-08$).

21 **The IGF like family receptor 1 is differentially expressed in BK virus nephropathy in** 22 **kidney transplant patients.**

23 We identified IGFLR1 as among the genes whose expression was most significantly
24 different when comparing stable kidney graft tissues from transplant patients to that of patients
25 with BK virus-positive transplant nephropathy (BKVN). When sorting each of the transcripts
26 measured in the kidneys of patients with BKVN as compared to that of patients with stable
27 kidney graft, IGFLR1 ranked 49 out of 54675 total transcripts (Table 3). Differential expression
28 of IGFLR1 in BK virus nephropathy was statistically significant (Table 3; $p=1.66E-07$).

We queried a second microarray dataset to determine if we could independently
validate IGFLR1 differential expression in BK virus nephropathy. We again identified IGFLR1 as
among the genes whose expression was most significantly different when comparing normal
kidney transplant biopsy to biopsy tissue from patients with BK virus nephropathy (BKVN).
When sorting each of the transcripts measured in the kidneys of patients with BKVN as
compared to that of patients with normal kidney transplant, IGFLR1 ranked 153 out of 29096
total transcripts (Table 4). Differential expression of IGFLR1 in BK virus nephropathy was again
statistically significant (Table 4; $p=1.52E-08$).

Expression of PSMB9 is significantly increased in BK virus nephropathy.

We obtained exact mRNA expression levels for PSMB9 in normal kidney transplant tissue and in BK virus nephropathy to determine the magnitude and direction of change in PSMB9 expression in patients with BK virus infection of the kidneys. In both datasets queried, PSMB9 expression was significantly higher in BKVN than in normal kidney transplant tissue (Figure 1 and Figure 2; $p < 0.0001$ and $p = 0.0002$, respectively). We measured a fold change of 1.258 ± 0.169 and 1.234 ± 0.027 in PSMB9 expression in BK virus nephropathy (Table 1 and Table 2, respectively).

Expression of IGFLR1 is significantly increased in BK virus nephropathy.

We also obtained exact mRNA expression levels for IGFLR1 in normal kidney transplant tissue and in BK virus nephropathy to determine the magnitude and direction of change in IGFLR1 expression in patients with BK virus infection of the kidneys. In both datasets queried, IGFLR1 expression was significantly higher in BKVN than in normal kidney transplant tissue (Figure 3 and Figure 4; $p < 0.0001$ and $p = 0.0044$, respectively). We measured a fold change of 1.285 ± 0.163 and 1.233 ± 0.042 in IGFLR1 expression in BK virus nephropathy (Table 3 and Table 4, respectively).

Next, we performed comparative transcriptome analysis using two independent microarray datasets from the primary tumors of patients with breast cancer to evaluate conservation of BK virus differential expression targets in human breast cancer.

The proteasomal subunit beta 9 is differentially expressed in the primary tumors of patients with breast cancer.

We identified PSMB9 as among the genes whose expression was most significantly different when comparing normal breast tissues to primary tumors from patients with breast cancer. When sorting each of the transcripts measured in primary tumors of the breast as compared to that of the normal breast (Table 5), PSMB9 ranked 4287 out of 22283 total transcripts, equating to 80.8% differential expression. Differential expression of PSMB9 in the primary tumors of patients with breast cancer was statistically significant (Table 5; $p = 2.02E-03$).

We queried a second microarray dataset to determine if we could independently validate PSMB9 differential expression in human breast cancer. We again identified PSMB9 as among the genes whose expression was most significantly different when comparing normal breast tissues to primary tumors from patients with breast cancer. When sorting each of the transcripts measured in primary tumors of the breast as compared to that of the normal breast, PSMB9 ranked 7717 out of 19076 total transcripts (Table 6), equating to 59.5% differential expression. Differential expression of PSMB9 in the primary tumors of patients with breast cancer was statistically significant (Table 6; $p = 1.24E-02$).

The IGF like family receptor 1 is differentially expressed in the primary tumors of patients with breast cancer.

We identified IGFLR1 as among the genes whose expression was most significantly different when comparing normal breast tissues to primary tumors from patients with breast

1 cancer. When sorting each of the transcripts measured in primary tumors of the breast as
2 compared to that of the normal breast, IGFLR1 ranked 5883 out of 22283 total transcripts
3 (Table 7), equating to 73.6% differential expression. Differential expression of IGFLR1 in the
primary tumors of patients with breast cancer was statistically significant (Table 7; $p=8.75E-03$).

4 We queried a second microarray dataset to determine if we could independently
5 validate IGFLR1 differential expression in human breast cancer; this dataset did not provide
measurement data for probes corresponding to IGFLR1.

6 Thus, we found here through blind, whole transcriptome comparative analysis kidney
7 transplant tissues, in patients with stable grafts and in patients with BK virus nephropathy,
8 significant differential expression and transcriptional induction of PSMB9 and IGFLR1.
9 Through integration with a second transcriptome analysis of primary tumors from patients with
breast cancer and normal breast tissue, we found conserved differential expression of PSMB9
and IGFLR1 in human breast cancer.

10 Discussion

11 We and others have identified similarities at the systems level between cancers induced
12 in the murine breast through modeling of viral infection such as integration events and viral
13 oncogene expression, including the SV40 polyomavirus large T-antigen, and murine mammary
14 tumor virus (MMTV) expression the polyomavirus T-antigen (MMTV-PyMT), but previously
15 studies have failed to identify the presence of viral DNA from the BK polyomavirus^{4,5}, a virus
16 with the potential to induce transformation^{15,17}. We argue that these failures are due to
17 limitations in technology utilized to measure the presence of virus in breast tissues. Viral
18 transformation can occur solely through oncoprotein expression and in the absence of
detectable capsid protein expression, virion production, leaving efforts to measure one viral
transcript as a surrogate of viral DNA futile. Here we utilized a systems level approach to
determine in an unbiased fashion whether transcription was altered by BK virus infection in any
way similar to that found in the primary tumors of patients with breast cancer, identifying two
conserved differential gene expression targets, PSMB9 and IGFLR1.

19 PSMB9 is a beta subunit for the 20S proteasome. One study has reported over-
20 expression of PSMB9 in early stage breast cancer and its association with risk of early relapse
21 and death. Single-nucleotide polymorphism rs2871921 at IGFLR1 has been reported as a cis-
expression quantitative trait locus (eQTL) in patients with breast cancer¹⁹.

22 These data do not provide evidence that BK virus is a transformative agent in breast
23 cancer but do provide rationale for more rigorous evaluation of the presence of BK viral
24 oncogene or oncoprotein expression through next-generation sequencing and proteomic
25 methods in primary tumors of the breast in humans. Importantly, such analyses would
26 illuminate the presence of any viral oncoproteins, not restricted to the BK polyomavirus. We
27 provide here evidence of significant conservation of transcriptional modulation of IGFLR1 and
28 PSMB9 in BK virus nephropathy and in human breast cancer, providing one piece of evidence
illustrating similarities between transcriptional programs of BK virus infection and human breast
cancer.

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Rank	ID	p-value	t	B	FC	Gene	Gene name
50	204279_at	1.71E-07	6.133374	7.158907	1.258 ± 0.169	PSMB9	proteasome subunit beta 9

Table 1: PSMB9 is differentially expressed in BK virus nephropathy.

Rank of differential expression, probe ID, p-value with respect to global differential expression, t, a moderated t-statistic, B, the log-odds of differential expression between the groups compared, fold change (FC) of PSMB9 expression in BKVN as compared to stable kidney graft tissue, gene and gene name are listed in this chart.

Rank	ID	p-value	t	B	FC	Gene	Gene name
145	8118571	1.29E-08	10.960513	10.20535	1.234 ± 0.027	PSMB9	proteasome subunit beta 9

Table 2: PSMB9 is differentially expressed in BK virus nephropathy.

Rank of differential expression, probe ID, p-value with respect to global differential expression, t, a moderated t-statistic, B, the log-odds of differential expression between the groups compared, fold change (FC) of PSMB9 expression in BKVN as compared to stable kidney graft tissue, gene and gene name are listed in this chart.

Rank	ID	p-value	t	B	FC	Gene	Gene name
49	219690_at	1.66E-07	6.142383	7.188193	1.285 ± 0.163	IGFLR1	IGF like family receptor 1

Table 3: IGFLR1 is differentially expressed in BK virus nephropathy.

Rank of differential expression, probe ID, p-value with respect to global differential expression, t, a moderated t-statistic, B, the log-odds of differential expression between the groups compared, fold change (FC) of IGFLR1 expression in BKVN as compared to stable kidney graft tissue, gene and gene name are listed in this chart.

Rank	ID	p-value	t	B	FC	Gene	Gene name
153	8036136	1.52E-08	10.828561	10.04332	1.233 ± 0.042	IGFLR1	IGF like family receptor 1

Table 4: IGFLR1 is differentially expressed in BK virus nephropathy.

Rank of differential expression, probe ID, p-value with respect to global differential expression, t, a moderated t-statistic, B, the log-odds of differential expression between the groups compared, fold change (FC) of IGFLR1 expression in BKVN as compared to stable kidney graft tissue, gene and gene name are listed in this chart.

Rank	ID	p-value	t	B	Gene	Gene name
4287	204279_at	2.02E-03	3.1479794	-1.9427	PSMB9	proteasome subunit beta 9

Table 5: PSMB9 is differentially expressed in human breast cancer.

Rank of differential expression, probe ID, p-value with respect to global differential expression, t, a moderated t-statistic, B, the log-odds of differential expression between the primary tumors of patients with breast cancer as compared to normal breast tissue, gene and gene name are listed in this chart.

Rank	ID	p-value	t	B	Gene	Gene name
7717	2903285	1.24E-02	2.59	-3.801855	PSMB9	Proteasome subunit beta type-9

Table 6: PSMB9 is differentially expressed in human breast cancer.

Rank of differential expression, probe ID, p-value with respect to global differential expression, t, a moderated t-statistic, B, the log-odds of differential expression between the primary tumors of patients with breast cancer as compared to normal breast tissue, gene and gene name are listed in this chart.

Rank	ID	p-value	t	B	Gene	Gene name
5883	219690_at	8.75E-03	2.6606074	-3.26241	IGFLR1	IGF like family receptor 1

Table 7: IGFLR1 is differentially expressed in human breast cancer.

Rank of differential expression, probe ID, p-value with respect to global differential expression, t, a moderated t-statistic, B, the log-odds of differential expression between the primary tumors of patients with breast cancer as compared to normal breast tissue, gene and gene name are listed in this chart.

Figure 1: Significant transcriptional induction of PSMB9 by BK virus infection in BKVN.

mRNA expression levels of PSMB9 in stable kidney graft tissue (left) and in BK virus nephropathy in patients with kidney transplant (right) is graphically depicted with mean mRNA expression levels marked and the results of a statistical test evaluating significance of difference in PSMB9 expression in BKVN, a p-value, listed above.

Figure 2: Significant transcriptional induction of PSMB9 by BK virus infection in BKVN.

mRNA expression levels of PSMB9 in stable kidney graft tissue (left) and in BK virus nephropathy in patients with kidney transplant (right) is graphically depicted with mean mRNA expression levels marked and the results of a statistical test evaluating significance of difference in PSMB9 expression in BKVN, a p-value, listed above.

Figure 3: Significant transcriptional induction of IGFLR1 by BK virus infection in BKVN.

mRNA expression levels of IGFLR1 in stable kidney graft tissue (left) and in BK virus nephropathy in patients with kidney transplant (right) is graphically depicted with mean mRNA expression levels marked and the results of a statistical test evaluating significance of difference in IGFLR1 expression in BKVN, a p-value, listed above.

Figure 4: Significant transcriptional induction of IGFLR1 by BK virus infection in BKVN.

mRNA expression levels of IGFLR1 in stable kidney graft tissue (left) and in BK virus nephropathy in patients with kidney transplant (right) is graphically depicted with mean mRNA expression levels marked and the results of a statistical test evaluating significance of difference in IGFLR1 expression in BKVN, a p-value, listed above.