Brief Report

Cancer-Related Ischemic Stroke Has a Distinct Blood mRNA Expression Profile

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Background and Purpose—Comorbid cancer is common in patients with acute ischemic stroke (AIS). As blood mRNA profiles can distinguish AIS mechanisms, we hypothesized that cancer-related AIS would have a distinctive gene expression profile.

Methods—We evaluated 4 groups of 10 subjects prospectively enrolled at 3 centers from 2009 to 2018. This included the group of interest with active solid tumor cancer and AIS and 3 control groups with active cancer only, AIS only, or vascular risk factors only. Subjects in the AIS-only and cancer-only groups were matched to subjects in the cancer-stroke group by age, sex, and cancer type (if applicable). Subjects in the vascular risk factor group were matched to subjects in the cancer-stroke and stroke-only groups by age, sex, and vascular risk factors. Blood was drawn 72 to 120 hours after stroke. Total RNA was processed using 3′ mRNA sequencing. ANOVA and Fisher least significant difference contrast methods were used to estimate differential gene expression between groups.

Results—In the cancer-stroke group, 50% of strokes were cryptogenic. All groups had differentially expressed genes that could distinguish among them. Comparing the cancer-stroke group to the stroke-only group and after accounting for cancer-only genes, 438 genes were differentially expressed, including upregulation of multiple genes/pathways implicated in autophagy signaling, immunity/inflammation, and gene regulation, including IL (interleukin)-1, interferon, relaxin, mammalian target of rapamycin signaling, SQSTMI1 (sequestosome-1), and CREB1 (cAMP response element binding protein-1).

Conclusions—This study provides evidence for a distinctive molecular signature in blood mRNA expression profiles of patients with cancer-related AIS. Future studies should evaluate whether blood mRNA can predict detection of occult cancer in patients with AIS.

Clinical Trial Registration—URL: https://clinicaltrials.gov. Unique identifier: NCT02604667. **(*Stroke*. 2019;50:00-00. DOI: 10.1161/STROKEAHA.119.026143.)**

Key Words: gene expression ■ humans ■ neoplasms ■ risk factors ■ stroke

About one-third of acute ischemic strokes (AISs) have no identifiable cause and are labeled cryptogenic.[1] Among patients with cryptogenic AIS, retrospective studies suggest that 5% to 10% will be diagnosed with cancer within 1 to 2 years after their stroke.[2,3] Additionally, a large claims-based analysis reported that in the year before cancer diagnosis, AIS risk is increased by 59%.[4] Therefore, some cryptogenic AISs are probably caused or triggered by occult cancer. This hypothesis is supported by numerous reports of cancer presenting with cryptogenic AIS.[5]

Because earlier detection of cancer might translate into better outcomes, having a noninvasive biomarker that reliably predicts detection of cancer in AIS patients could be useful. Previous studies have demonstrated that differential blood mRNA expression profiles can distinguish AIS subtypes, hemorrhagic versus ischemic stroke, and transient ischemic attack versus mimics.[6] We hypothesized that cancer-related AIS would also have a distinct blood mRNA expression profile.

Methods

Design

This analysis included 40 subjects prospectively enrolled at academic centers in New York, California, and Alberta, Canada, between 2009 and 2018. Subjects were divided into 4 groups of 10,

Received April 29, 2019; final revision received July 19, 2019; accepted July 26, 2019.

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Guest Editor for this article was Harold P. Adams, MD.

The online-only Data Supplement is available with this article at https://www.ahajournals.org/doi/suppl/10.1161/STROKEAHA.119.026143.

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including the group of interest, subjects with active solid tumor cancer, and AIS. The 3 control groups were subjects with active cancer only, AIS only, or vascular risk factors only (VRFC). Subjects in the stroke-only group were individually matched to subjects in the cancer-stroke group by age stratum (≥65 versus <65 years) and sex, whereas subjects in the cancer-only group were individually matched to subjects in the cancer-stroke group by age stratum (≥65 versus <65 years), sex, and cancer type. Subjects in the VRFC group were individually matched to subjects in the cancer-stroke and stroke-only groups by age stratum (≥65 versus <65 years), sex, and several vascular risk factors, including race. In cases when an exact match on all vascular risk factors could not be identified, the most similar available subject was used for matching. Full eligibility/matching criteria are described in Methods (online-only Data Supplement). Participating institutions' review boards approved this study. This study was registered at https://clinicaltrials.gov (unique identifier: NCT02604667). All subjects or their surrogates provided written informed consent. Study data and materials are available on reasonable request.

Sample Processing

Blood was collected in PAXgene tubes (Qiagen). Total RNA was isolated per PreAnalytix protocol. In the stroke groups, blood was drawn 72 to 120 hours from onset. QuantSeq FWD 3′ mRNA (Lexogen) libraries were prepared and globin depleted. Unique molecular identifiers were incorporated to collapse polymerase chain reaction duplicates. Samples were sequenced to an average 7.4 million SE 100 bp reads. Data were aligned (STAR 2.5.3c, Hg38, GENCODE 25), and gene-level expression was quantified. Reads were quantile normalized after an offset of 0.0001 and log transformation. Genes with ≤10 reads across all samples were excluded, yielding 12 043 genes for analysis.

Analysis

Subject groups were compared using ANOVA in Partek Genomics Suite (Partek, Inc). To delineate unique and common expression patterns between the cancer-stroke and stroke-only groups, while accounting for the contribution of cancer through the cancer-only group, the gene-level expression of each subject group was compared with the VRFC group or to each other. Genes with a false discovery rate–corrected value of $P<0.2$ (nominal $P<0.05$) and a fold change >1.2 were considered significant. This fold-change criterion was used to promote identification of biologically significant gene expression level change. Differentially expressed genes were then overlapped to determine whether the cancer-stroke group had a distinct expression profile. The restricted maximum likelihood method of variance estimates for unbalanced designs and Fisher least significant difference contrast method were used to estimate differential gene expression between subject groups. For data visualization, we performed unsupervised hierarchical clustering and principal component analysis. Ingenuity Pathway Analysis identified overrepresented pathways (see Methods in the online-only Data Supplement for details).

Results

Characteristics

Demographic and vascular risk factors were similar between groups (Table). In the cancer-stroke group, underlying cancers were lung (n=3), breast (n=3), prostate (n=2), pancreas (n=1), or ovarian (n=1); adjudicated stroke mechanisms were cryptogenic (n=5) or cardioembolic (n=5).

Differential Expression Compared With VRFC Group

Between the cancer-stroke and VRFC groups, there were 1797 differentially expressed genes (867 upregulated and 930 downregulated; Table I in the online-only Data Supplement). Principal component analysis and unsupervised hierarchical clustering separated most subjects in these groups based on their differentially expressed genes (Figure 1A and 1B). There were 102 pathways overrepresented (Table II in the online-only Data Supplement), including the activation of 9 signaling pathways (Figure IA in the online-only Data Supplement).

Between the stroke-only and VRFC groups, there were 1197 differentially expressed genes (732 upregulated and 465 downregulated; Table III in the online-only Data Supplement). Principal component analysis and unsupervised hierarchical clustering separated most subjects in these groups based on their differentially expressed genes (Figure 1C and 1D). There were 76 pathways overrepresented (Table IV in the online-only Data Supplement), including many pathways that we previously identified as regulated following stroke (Figure IA in the online-only Data Supplement).[7]

Differential gene expression between the cancer-only and VRFC groups is described in Results in the online-only Data Supplement, Tables V and VI in the online-only Data Supplement, and Figure II in the online-only Data Supplement.

Unique Differential Expression in Cancer-Stroke and Stroke-Only Groups

When overlapping differentially expressed genes from all 4 groups, there were 1323 uniquely differentially expressed genes in the cancer-stroke group, 687 uniquely differentially expressed genes in the stroke-only group, and 342 differentially expressed genes in common between the cancer-stroke and stroke-only groups (Figure IB in the online-only Data Supplement; Tables VII through IX in the online-only Data Supplement). Figure IC in the online-only Data Supplement shows the 10 most significantly overrepresented pathways.

Comparison of Cancer-Stroke and Stroke-Only Transcriptomes

There were 448 differentially expressed genes between the cancer-stroke and stroke-only groups including 205 with higher expression in the cancer-stroke group and 243 with higher expression in the stroke-only group (Table X in the online-only Data Supplement). Principal component analysis and unsupervised hierarchical clustering separated cancer-stroke subjects from stroke-only subjects based on their differentially expressed genes (Figure 2A and 2B). Most (n=438) of the 448 differentially expressed genes between the cancer-stroke and stroke-only groups did not overlap with the differentially expressed genes between the cancer-only and VRFC groups. These data along with the uniquely overrepresented pathways are provided in Figure 2C, Results in the online-only Data Supplement, and Table XI in the online-only Data Supplement.

Expression Patterns According to Stroke Mechanism Among Cancer-Stroke Patients

In a post hoc analysis comparing the mRNA expression patterns between the 5 cancer-stroke patients with adjudicated

Table. Subject Characteristics, Stratified by Study Group

Characteristics*	Cancer-Stroke (n=10)	Stroke Only (n=10)	Cancer Only (n=10)	Vascular Risk Factors Only (n=10)
Age, y; mean (SD)	63 (12)	67 (15)	59 (18)	61 (11)
Women	6 (60)	6 (60)	6 (60)	6 (60)
Race				
White	7 (70)	10 (100)	7 (70)	7 (70)
Black	2 (20)	0 (0)	3 (30)	1 (10)
Other	1 (10)	0 (0)	0 (0)	2 (20)
Time from last known well to blood draw, h (mean±SD)	94 (11)	95 (12)	NA	NA
Cancer type				
Lung	3 (30)	NA	3 (30)	NA
Breast	3 (30)	NA	3 (30)	NA
Prostate	2 (20)	NA	3 (30)	NA
Pancreas	1 (10)	NA	0 (0)	NA
Ovarian	1 (10)	NA	1 (10)	NA
Adenocarcinoma	10 (100)	NA	10 (100)	NA
Systemic metastases	9 (90)	NA	10 (100)	NA
Brain metastases	1 (10)	NA	3 (30)	NA
Chemotherapy within 30 d	7 (70)	NA	8 (80)	NA
WBC, count/nL; mean (SD)	7.7 (3.8)	7.0 (2.6)	5.9 (1.9)	…†
Platelet, count/nL; mean (SD)	197 (48)	207 (63)	270 (110)	…†
Stroke mechanism‡				
Large artery atherosclerosis	0 (0)	1 (10)	NA	NA
Small vessel disease	0 (0)	1 (10)	NA	NA
Cardioembolic	5 (50)	2 (20)	NA	NA
Other	0 (0)	1 (1)	NA	NA
Cryptogenic	5 (50)	5 (50)	NA	NA
NIH Stroke Scale, mean (SD)	4 (3)	1 (2)	NA	NA
Vascular risk factors				
Diabetes mellitus	2 (20)	0 (0)	2 (20)	1 (10)
Hypertension	5 (50)	7 (70)	4 (40)	7 (70)
Hyperlipidemia	2 (20)	6 (60)	1 (10)	4 (40)
Peripheral artery disease	0 (0)	0 (0)	1 (10)	0 (0)
Atrial fibrillation	1 (10)	0 (0)	1 (10)	0 (0)
Coronary artery disease	0 (0)	0 (0)	1 (10)	0 (0)
Chronic kidney disease	0 (0)	0 (0)	1 (10)	0 (0)
Prior stroke/TIA	2 (20)	0 (0)	1 (10)	0 (0)
Smoking history (any)	3 (30)	2 (20)	4 (40)	4 (40)

NA indicates not applicable; NIH, National Institutes of Health; TIA, transient ischemic attack; TOAST, Trial of ORG 10172; and WBC, white blood cell.
*All data are presented as number (%) unless otherwise specified.
†Not performed because samples amenable to blood count calculation were not collected.
‡According to the TOAST classification.

cardioembolic mechanisms and the 5 cancer-stroke patients with adjudicated cryptogenic mechanisms, there were similar expression profiles with only 15 differentially expressed genes between groups.

Discussion

In this prospective pilot study, we found that cancer patients with AIS have distinctive blood gene expression profiles as compared with stroke-only and cancer-only controls. We

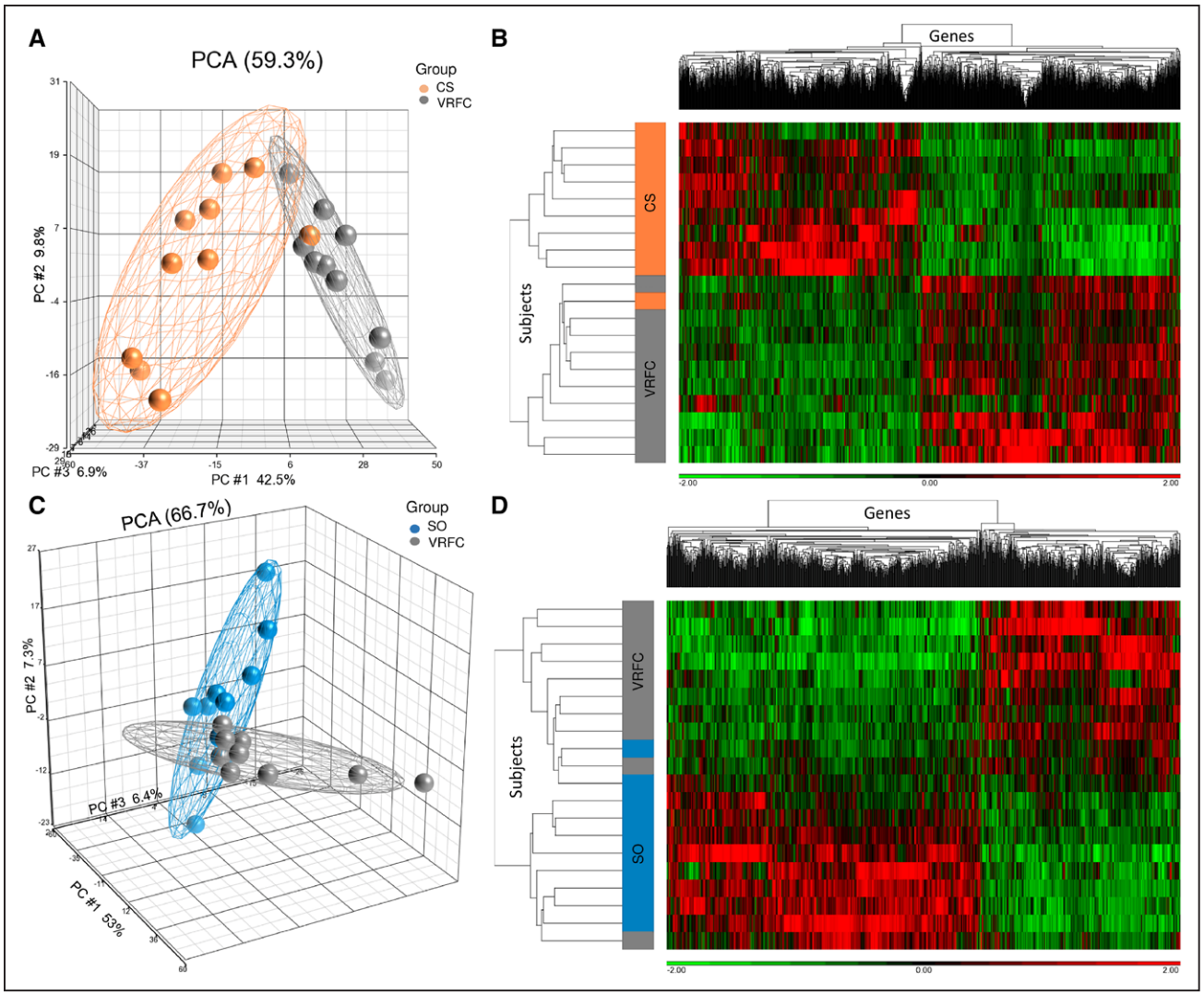

Figure 1. Differential RNA expression compared to the vascular risk factor control group. **A** and **B**, Principal component analysis (PCA) and unsupervised hierarchical clustering of 1797 differentially expressed genes between the cancer-stroke (CS) and vascular risk factor control (VRFC) groups. **C** and **D**, PCA and unsupervised hierarchical clustering of 1197 differentially expressed genes between the stroke-only (SO) and VRFC groups. Orange denotes CS group; gray denotes VRFC group; and blue denotes SO group. Red represents high expressed genes; green represents low expressed genes. PC indicates principal components.

identified 448 differentially expressed genes that differentiated between the cancer-stroke and stroke-only patients, as well as hundreds of shared genes/pathways between the 2 groups, indicating both common and cancer-specific responses to stroke.

Pathways unique to cancer-stroke patients included autophagy, IL (interleukin; IL-1, IL-10, and IL-12) signaling, ATM (ataxia telangiectasia mutated protein) signaling, base excision repair, helper T-cell (Th1, Th2, and Th17) activity, phagosome formation, pattern recognition receptors signaling, TREM1 signaling, and neuroinflammation signaling. These pathways primarily involve inflammation, cancer formation/progression, transcriptional regulation, cortical circuit plasticity, and the hypoxia response. For instance, ATM is a key regulator of signaling cascades that respond to DNA strand breaks and thereby functions as a caretaker, suppressing tumorigenesis in specific T-cell lineages.[8] The base excision repair pathway—a target for cancer treatment—was also overrepresented in the cancer-stroke group. Immune responses, including helper T-cell activity, play important roles in tumorigenesis by enabling immune escape of malignantly transformed cells.[9] Additionally, tumors often develop at sites of chronic inflammation, and inflammation can promote tumor progression and increase thrombotic risk.[10] Therefore, modulation of IL, helper T-cell, and TREM1 pathways are consistent with this. Additionally, major transcriptional regulators were differentially expressed between the cancer-stroke and stroke-only groups, including CREB1 (cAMP response element binding protein-1) and SQSTM1 (sequestosome-1). CREB1—a CREB transcription factor, which was downregulated in the cancer-stroke group versus the stroke-only group—controls cortical circuit plasticity and functional recovery after stroke; and increased levels of CREB expression enhance stroke recovery, while blocking CREB signaling hinders recovery.[11] SQSTM1 (p62), which was upregulated in the cancer-stroke group versus the stroke-only groups, is a critical regulator of the hypoxia response, NF-kB, and TNF (tumor necrosis factor) signaling.[12]

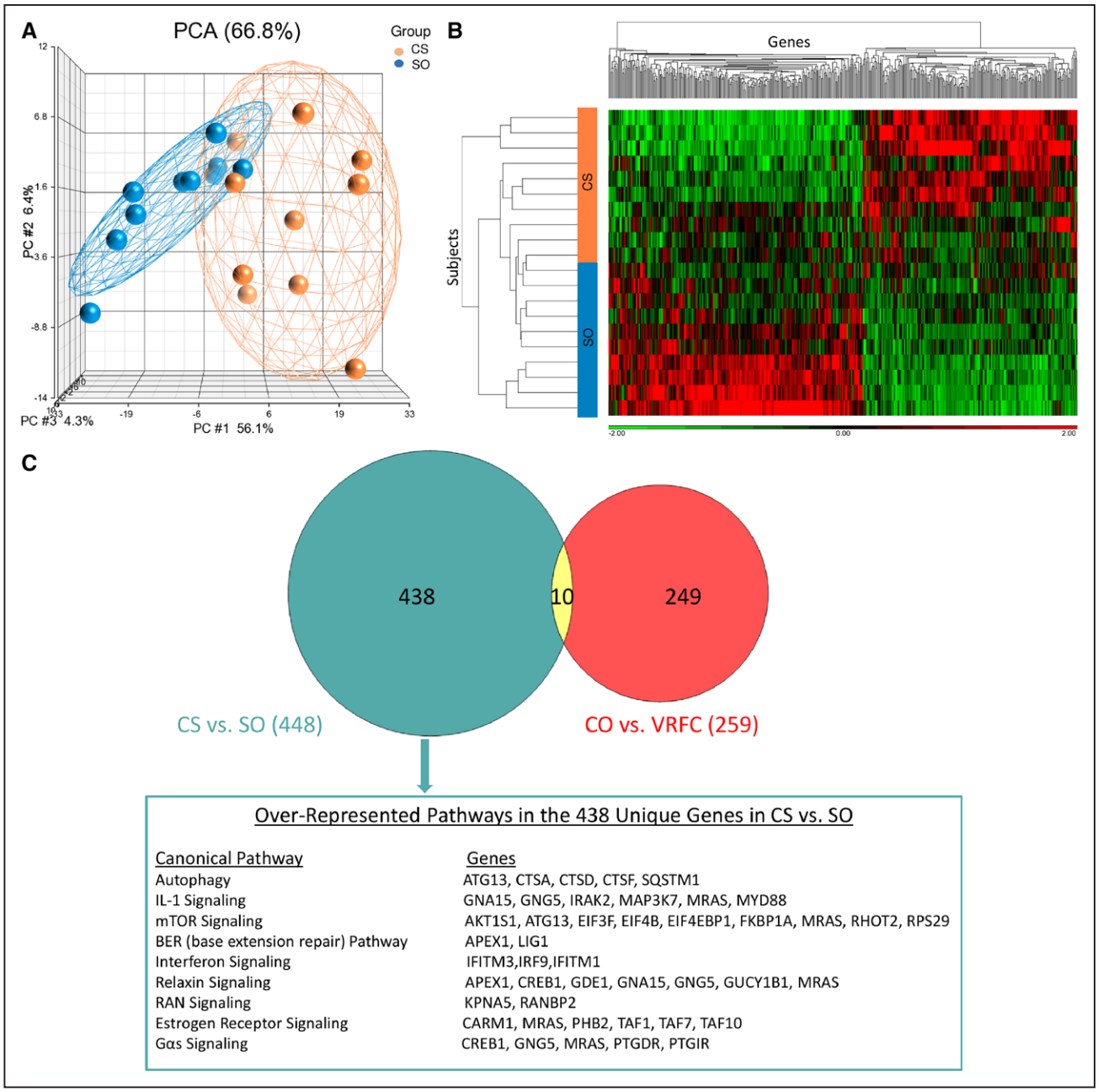

Figure 2. Comparison of the cancer-stroke and stroke-only transcriptomes. **A** and **B**, Principal component analysis (PCA) and unsupervised hierarchical clustering of 448 differentially expressed genes between the cancer-stroke (CS) and stroke-only (SO) groups. Orange denotes CS group; blue denotes SO group. Red represents high expressed genes; green represents low expressed genes. **C**, Overlap between the differentially expressed genes in the CS vs SO groups and the cancer-only (CO) vs the vascular risk factor control (VRFC) groups. The table represents the canonical pathways that are significantly overrepresented when comparing CS subjects to SO subjects. IL indicates interleukin; Gαs, G-s alpha subunit; mTOR, mamallian target of rapamycin; PC, principal components; and RAN, RAs-related nuclear.

Although limited by a small and heterogenous sample size with a large false-positive rate, this study suggests that RNA gene signatures in blood obtained 72 to 120 hours after AIS onset can discern AIS associated with cancer. As 5% to 10% of patients with cryptogenic AIS are diagnosed with cancer soon after their stroke,[2,3] these data indicate that RNA expression profiling might be a useful noninvasive tool to screen these patients for cancer. However, before entering clinical use, external validation in a larger cohort is required. Future studies should assess how RNA gene profiling can delineate pathophysiological mechanisms among cancer-stroke patients.

Sources of Funding

This study was supported by National Institutes of Health grants K23NS091395, R01NS097000, and R01NS106950.

Disclosures

Dr Kamel serves as co-principal investigator for the National Institutes of Health (NIH)–funded ARCADIA trial (Atrial Cardiopathy and Antithrombotic Drugs in Prevention After Cryptogenic Stroke), which receives in-kind study drug from BMS-Pfizer and in-kind study assays from Roche Diagnostics; the Deputy Editor for *JAMA Neurology*; a steering committee member for Medtronic Stroke AF trial; an end point adjudication committee member for a trial of empagliflozin

for Boehringer-Ingelheim; and has served on an advisory board for Roivant Sciences related to Factor XI inhibition. Dr Tagawa has received grants and personal fees from Sanofi, Astellas, Janssen, Bayer, AbbVie, Amgen, Clovis, and Endocyte and grants from BMS, Lily, Novartis, Merck, Millennium, and AstraZeneca. Dr Saxena has received personal fees from AstraZeneca, Medtronic, Lily Oncology, Takeda, Guardant Health, and Genentech. Dr Iadecola has served on the scientific advisory board for Broadview Ventures. Dr DeAngelis has received personal fees for serving on scientific advisory boards for Sapience Therapeutics, Tocagen, and Roche. Dr Elkind has received royalties from UpToDate for a chapter on cryptogenic stroke and serves as co-principal investigator for the NIH-funded ARCADIA trial, which receives in-kind study drug from BMS-Pfizer and support for laboratory assays from Roche Diagnostics. Drs Jickling, Sharp, and Stamova hold a patent on gene expression signatures for predicting stroke and its causes; Dr Sharp also has received NIH grants regarding stroke. The other authors report no conflicts.

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