SUPPLEMENTAL MATERIAL

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

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Supplemental Methods

Study Eligibility Criteria and Subject Matching

Subjects in the cancer-stroke, stroke-only, and cancer-only groups included in this analysis were recruited from 2016-2018 at Weill Cornell Medicine and Memorial Sloan Kettering Cancer Center as part of the ongoing, prospective Mechanisms Of Ischemic STroke in Cancer (MOST-Cancer) study. The eligibility criteria for the MOST-Cancer study are as follows:

Inclusion Criteria:

- 18 years of age or older
- Active solid tumor cancer per standard definition (only for the cancer-stroke and cancer-only groups)[1]
- MRI confirmed acute ischemic stroke (only for the cancer-stroke and stroke-only groups)
- Available for blood draw at 96 hours (+/- 24 hours) from last known well time (only for the cancer-stroke and stroke-only groups) or within 2 weeks of enrollment (only for the cancer-only group)
- Available for Transcranial Doppler within 2 weeks of enrollment

Exclusion Criteria:

- Primary brain tumor or hematological cancer
- Treatment with intravenous or intraarterial thrombolysis or mechanical thrombectomy
- Platelets <50,000/mm^3
- Hemodialysis within 14 days
- Active pregnancy
- Infection within 14 days per Infectious Diseases Society of America (IDSA) criteria[2]

Subjects in the stroke-only group were prospectively individually matched to subjects in the cancer-stroke group by age stratum (≥65 years vs. <65 years) and sex; while subjects in the cancer-only group were prospectively individually matched to subjects in the cancer-stroke group by age stratum (≥65 years vs. <65 years), sex, and cancer type. For one cancer-stroke subject with pancreatic cancer, an appropriate age- and sex-matched cancer-only control with pancreatic cancer could not be recruited. Therefore, this subject was instead matched to an age- and sex-matched control with prostate cancer.

Subjects in the vascular risk factor control group were prospectively recruited at the University of California, Davis from 2009-2011. The eligibility criteria used to recruit these subjects has been described previously.[3] These subjects were individually matched to subjects in the cancer-stroke and stroke-only groups by age stratum (≥65 years vs. <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.

Adjudication of Index Stroke Mechanisms

A single vascular neurologist (BBN) adjudicated stroke mechanisms (for the cancer-stroke and stroke-only groups) at the time of hospital discharge using the TOAST criteria.[4]

Principal Component Analysis Methodology

Principal component analysis (PCA) is a data dimensionality reduction technique, where one sphere represents one subject, and the position of the sphere on the PCA map is determined by the collective expression of the differentially expressed genes under consideration. Correlation, where all variables have equal influence on the principal components, was used for scaling of variables.

Assessment of the Potential Biological Significance of Differentially Expressed Genes

Using Ingenuity Pathway Analysis (IPA®, Qiagen), gene identifiers from each differentially expressed gene list were overlaid onto a global molecular network developed from information contained in the Ingenuity Knowledge Database for canonical pathways. Canonical Pathway Analysis was used to identify the pathways from the IPA library of canonical pathways that were most significant to the gene list. Fisher's exact test was used to calculate a p-value for the probability that each canonical pathway assigned to that dataset was due to chance alone. A p-value of <0.05 was considered statistically significant for overrepresentation of the molecules in a given pathway. IPA was also used to predict whether pathways overrepresented with differentially expressed genes were activated or inhibited. IPA with a Z-score algorithm was used to compare the uploaded dataset of differentially expressed genes with canonical pathway patterns, based on IPA's curated literature findings, the activation state of one or more key molecules when a pathway was activated, and the molecules' causal relationships with each other. Pathway activation was defined as a Z-score >2.0 and pathway inhibition was defined as a Z-score <-2.0.

Supplemental Results

Differential Expression in the Cancer-Only Group

Between the cancer-only and VRFC groups, there were 259 differential expressed genes (96 upregulated, 163 downregulated) (Supplemental Table 6). Principal component analysis and unsupervised hierarchical clustering separated most subjects in these 2 groups based on their differentially expressed genes (Supplemental Figure 1). There were 59 pathways overrepresented with differentially expressed genes (Supplemental Table 7). This included several pathways that are inherent or implicated in cancer, including breast cancer regulation by stathmin1, prostate cancer signaling, acute myeloid leukemia signaling, chronic myeloid leukemia signaling, ERK/MAPK signaling, and PI3K/AKT3 signaling.

Comparison of the Cancer-Stroke and Stroke-Only Transcriptomes

To account for the contribution of active cancer to gene expression in the cancer-stroke group, we overlapped the differentially expressed genes between the cancer-stroke and stroke-only groups with the differentially expressed genes between the cancer-only and vascular risk factor groups. After doing so, there were 438 unique differentially expressed genes between the cancer-stroke and stroke-only groups, including 201 with higher expression in the cancer-stroke group and 237 with higher expression in the stroke-only group (Supplemental Table 12). The 10 differentially expressed genes that overlapped between the cancer-stroke and cancer-only groups were ALDOA, ASH1L-AS1, BRWD1, EXOSC8, GPBP1, GTF3C6, KRCC1, MED18, PSMB1, RASA2. Many of these genes are implicated in cancer pathogenesis.[5-7] The overrepresented canonical pathways in the 438 unique differentially expressed genes between the cancer-stroke and stroke-only groups included autophagy, interleukin-1 signaling, mTOR Signaling, base excision repair pathway, interferon signaling, relaxin signaling, RAN signaling, estrogen receptor signaling, and GAS signaling.

Supplemental Table I. Differential Gene Expression between Cancer-Stroke Group and Vascular Risk Factor Control Group. See table in separate file.

Supplemental Table II. Overrepresented Pathways between Cancer-Stroke Group and Vascular Risk Factor Control Group

Ingenuity Canonical Pathways	**-Log (P-value)**	**Z-score**
Interferon Signaling	6.47	2.50
Th1 and Th2 Activation Pathway	4.63	*
RAR Activation	4.39	*
Th1 Pathway	4.29	0.23
ATM Signaling	4.15	0.24
NF-κB Signaling	4.14	1.10
Glucocorticoid Receptor Signaling	4.13	*
Inflammasome pathway	4.03	2.83
Phospholipase C Signaling	3.78	0.37
BER pathway	3.78	*
Toll-like Receptor Signaling	3.57	3.32
Phagosome Formation	3.48	*
IL-10 Signaling	3.47	*
TREM1 Signaling	3.40	2.84
iNOS Signaling	3.23	1.51
B Cell Receptor Signaling	3.21	0.78
PI3K Signaling in B Lymphocytes	3.10	0.22
Role of Pattern Recognition Receptors in Recognition of Bacteria and Viruses	3.01	2.36
Dendritic Cell Maturation	2.99	1.80
DNA Methylation and Transcriptional Repression Signaling	2.93	*
Systemic Lupus Erythematosus Signaling	2.90	*
T Cell Exhaustion Signaling Pathway	2.70	0.20
Production of Nitric Oxide and Reactive Oxygen Species in Macrophages	2.68	1.13
Tec Kinase Signaling	2.63	-1.34
Role of NFAT in Regulation of the Immune Response	2.60	-0.20
Sirtuin Signaling Pathway	2.60	2.27
IL-4 Signaling	2.51	*
Activation of IRF by Cytosolic Pattern Recognition Receptors	2.50	1.73
PPARα/RXRα Activation	2.45	0.00
iCOS-iCOSL Signaling in T Helper Cells	2.45	-1.81
Th2 Pathway	2.41	-0.73
Sumoylation Pathway	2.37	1.16
Role of BRCA1 in DNA Damage Response	2.34	0.00
Estrogen Receptor Signaling	2.26	*
Molecular Mechanisms of Cancer	2.24	*
PI3K/AKT Signaling	2.18	1.21
ILK Signaling	2.16	1.00
Role of Macrophages, Fibroblasts and Endothelial Cells in RA	2.15	*
Granzyme B Signaling	2.15	0.00
IL-15 Signaling	2.14	*
Wnt/Ca+ pathway	2.04	-1.51
p53 Signaling	2.03	0.00
AMPK Signaling	2.00	2.07
Antioxidant Action of Vitamin C	2.00	-0.58
PKCθ Signaling in T Lymphocytes	1.95	-0.89
Telomerase Signaling	1.92	0.00
Type I Diabetes Mellitus Signaling	1.92	1.60

Hypoxia Signaling in the Cardiovascular System	1.91	*
Assembly of RNA Polymerase III Complex	1.91	*
Integrin Signaling	1.85	-0.39
Acute Phase Response Signaling	1.85	1.61
B Cell Activating Factor Signaling	1.81	0.00
HMGB1 Signaling	1.78	0.00
Assembly of RNA Polymerase II Complex	1.75	*
Amyloid Processing	1.75	0.00
Role of RIG1-like Receptors in Antiviral Innate Immunity	1.75	1.41
Ascorbate Recycling (Cytosolic)	1.73	*
Regulation of IL-2 Expression in Activated and Anergic T Lymphocytes	1.73	*
CXCR4 Signaling	1.69	-0.23
Apoptosis Signaling	1.69	0.54
Neuroinflammation Signaling Pathway	1.68	3.55
Calcium-induced T Lymphocyte Apoptosis	1.67	-2.12
T Helper Cell Differentiation	1.66	*
DNA Double-Strand Break Repair by Homologous Recombination	1.66	*
FLT3 Signaling in Hematopoietic Progenitor Cells	1.61	-0.83
Th17 Activation Pathway	1.61	0.58
T Cell Receptor Signaling	1.61	*
Nur77 Signaling in T Lymphocytes	1.60	*
autophagy	1.60	*
Protein Ubiquitination Pathway	1.60	*
IL-12 Signaling and Production in Macrophages	1.58	*
Vitamin-C Transport	1.55	*
p38 MAPK Signaling	1.55	2.32
Endocannabinoid Cancer Inhibition Pathway	1.55	3.13
Natural Killer Cell Signaling	1.54	*
CTLA4 Signaling in Cytotoxic T Lymphocytes	1.52	*
G Protein Signaling Mediated by Tubby	1.51	*
tRNA Charging	1.51	-1.89
CD28 Signaling in T Helper Cells	1.51	-1.60
IL-1 Signaling	1.51	1.27
Hereditary Breast Cancer Signaling	1.50	*
Prostate Cancer Signaling	1.49	*
Death Receptor Signaling	1.47	0.83
Altered T Cell and B Cell Signaling in RA	1.46	*
April Mediated Signaling	1.46	-0.38
Netrin Signaling	1.45	*
Uracil Degradation II (Reductive)	1.45	*
Glutathione Redox Reactions II	1.45	*
Thymine Degradation	1.45	*
NER Pathway	1.45	-1.16
IL-17 Signaling	1.44	*
IGF-1 Signaling	1.41	-0.54
Role of JAK1, JAK2 and TYK2 in Interferon Signaling	1.40	0.00
TCA Cycle II (Eukaryotic)	1.40	-0.45
Insulin Receptor Signaling	1.40	-0.24
Regulation of Actin-based Motility by Rho	1.39	-0.58
NF-κB Activation by Viruses	1.38	-0.28
Role of PKR in Interferon Induction and Antiviral Response	1.35	*

Communication between Innate and Adaptive Immune Cells	1.33	*
IL-6 Signaling	1.32	1.70
Glioma Invasiveness Signaling	1.31	-0.30
Thrombin Signaling	1.31	-1.70

Abbreviations: RA, rheumatoid arthritis.
*The Z-score for the activation/suppression status could not be predicted.

Supplemental Table III. Differential Gene Expression between Stroke-Only Group and Vascular Risk Factor Control Group. See table in separate file.

Supplemental Table IV. Overrepresented Pathways between Stroke-Only Group and Vascular Risk Factor Control Group

Ingenuity Canonical Pathways	-Log (P-value)	Z-score
Oxidative Phosphorylation	5.55	0.23
Sirtuin Signaling Pathway	5.34	0.73
Mitochondrial Dysfunction	5.02	*
Protein Ubiquitination Pathway	3.56	*
CD40 Signaling	3.46	-1.16
iNOS Signaling	3.31	0.71
PI3K/AKT Signaling	3.30	2.18
RANK Signaling in Osteoclasts	2.92	-0.83
Role of JAK1, JAK2 and TYK2 in Interferon Signaling	2.84	0.00
TCA Cycle II (Eukaryotic)	2.84	-0.82
Glucocorticoid Receptor Signaling	2.68	*
Toll-like Receptor Signaling	2.64	1.34
B Cell Receptor Signaling	2.49	-0.69
TNFR2 Signaling	2.39	0.00
CD28 Signaling in T Helper Cells	2.37	-1.07
Estrogen Receptor Signaling	2.22	*
Regulation of IL-2 Expression in Activated and Anergic T Lymphocytes	2.22	*
Integrin Signaling	2.20	-1.61
RAR Activation	2.20	*
PPARα/RXRα Activation	2.19	-0.50
CD27 Signaling in Lymphocytes	2.19	-0.71
PPAR Signaling	2.18	0.00
N-acetylglucosamine Degradation I	2.07	*
NER Pathway	2.04	-0.91
fMLP Signaling in Neutrophils	2.02	-1.39
Production of Nitric Oxide and Reactive Oxygen Species in Macrophages	2.01	1.15
T Cell Receptor Signaling	2.00	*
Protein Kinase A Signaling	1.99	0.19
PI3K Signaling in B Lymphocytes	1.94	-0.54
IL-17 Signaling	1.93	*
Gαq Signaling	1.88	-0.78
Regulation of eIF4 and p70S6K Signaling	1.83	1.13
2-ketoglutarate Dehydrogenase Complex	1.79	*
Glutathione Redox Reactions II	1.79	*
N-acetylglucosamine Degradation II	1.79	*
Regulation of Actin-based Motility by Rho	1.79	-0.63
Acute Myeloid Leukemia Signaling	1.77	-1.41
D-myo-inositol (1,4,5,6)-Tetrakisphosphate Biosynthesis	1.75	0.54
D-myo-inositol (3,4,5,6)-Tetrakisphosphate Biosynthesis	1.75	0.54
3-phosphoinositide Degradation	1.74	0.78
UDP-N-acetyl-D-galactosamine Biosynthesis II	1.73	*
G Protein Signaling Mediated by Tubby	1.67	*
Pyridoxal 5'-phosphate Salvage Pathway	1.66	0.00
Role of PKR in Interferon Induction and Antiviral Response	1.66	*
D-myo-inositol-5-phosphate Metabolism	1.65	0.78
Insulin Receptor Signaling	1.63	-0.54
Telomerase Signaling	1.63	1.00

Autophagy	1.63	*
CDK5 Signaling	1.60	-2.11
IL-1 Signaling	1.60	0.00
Superpathway of Inositol Phosphate Compounds	1.60	0.00
Role of NFAT in Regulation of the Immune Response	1.57	-0.78
Antioxidant Action of Vitamin C	1.57	-0.33
Death Receptor Signaling	1.57	0.33
Unfolded protein response	1.55	*
Ephrin Receptor Signaling	1.55	-0.83
IL-17A Signaling in Airway Cells	1.54	-0.33
iCOS-iCOSL Signaling in T Helper Cells	1.53	-1.27
Apoptosis Signaling	1.48	0.00
IL-6 Signaling	1.48	-0.28
MIF-mediated Glucocorticoid Regulation	1.46	0.45
Sumoylation Pathway	1.46	1.89
Role of IL-17A in Arthritis	1.46	*
EGF Signaling	1.46	-1.41
Cardiac Hypertrophy Signaling	1.44	0.23
nNOS Signaling in Neurons	1.44	-1.00
Actin Cytoskeleton Signaling	1.43	-2.36
3-phosphoinositide Biosynthesis	1.42	0.24
GDP-mannose Biosynthesis	1.42	*
IL-17A Signaling in Fibroblasts	1.41	*
PDGF Signaling	1.40	-1.90
NF-κB Signaling	1.38	0.78
Interferon Signaling	1.36	0.45
TNFR1 Signaling	1.36	-0.82
Telomere Extension by Telomerase	1.35	*
CTLA4 Signaling in Cytotoxic T Lymphocytes	1.35	*

*The Z-score for the activation/suppression status could not be predicted.

Supplemental Table V. Differential Gene Expression between Cancer-Only Group and Vascular Risk Factor Control Group. See table in separate file.

Supplemental Table VI. Overrepresented Pathways between Cancer-Only Group and Vascular Risk Factor Control Group

Ingenuity Canonical Pathways	-Log (P-value)	Z-score
Estrogen Receptor Signaling	3.61	*
Breast Cancer Regulation by Stathmin1	3.55	*
Insulin Receptor Signaling	3.42	-1.41
Production of Nitric Oxide and Reactive Oxygen Species in Macrophages	3.16	0.00
PPAR Signaling	2.89	0.82
Integrin Signaling	2.88	-2.12
CDK5 Signaling	2.75	0.00
NAD Biosynthesis from 2-amino-3-carboxymuconate Semialdehyde	2.67	*
Dopamine Receptor Signaling	2.62	*
HIPPO signaling	2.43	*
Erythropoietin Signaling	2.34	*
CD28 Signaling in T Helper Cells	2.29	-1.63
Acute Myeloid Leukemia Signaling	2.15	-1.00
Cardiac β-adrenergic Signaling	2.13	1.34
Sirtuin Signaling Pathway	2.08	0.82
Oxidative Phosphorylation	2.04	-2.24
NAD biosynthesis II (from tryptophan)	1.97	*
IL-2 Signaling	1.93	*
Acetyl-CoA Biosynthesis III (from Citrate)	1.91	*
Colanic Acid Building Blocks Biosynthesis	1.91	*
ERK/MAPK Signaling	1.89	0.38
Dopamine-DARPP32 Feedback in cAMP Signaling	1.87	0.82
Apelin Endothelial Signaling Pathway	1.71	-1.34
IL-9 Signaling	1.71	*
IL-15 Signaling	1.71	*
PI3K/AKT Signaling	1.70	0.45
Regulation of Actin-based Motility by Rho	1.69	-2.00
Ephrin Receptor Signaling	1.68	-2.24
Growth Hormone Signaling	1.67	0.00
Iron homeostasis signaling pathway	1.65	*
UDP-D-xylose and UDP-D-glucuronate Biosynthesis	1.61	*
Inflammasome pathway	1.61	*
IL-3 Signaling	1.61	-1.00
Androgen Signaling	1.60	*
Neuregulin Signaling	1.59	*
JAK/Stat Signaling	1.59	-1.00
Glucocorticoid Receptor Signaling	1.58	*
Aryl Hydrocarbon Receptor Signaling	1.57	-0.45
IL-4 Signaling	1.56	*
RAR Activation	1.56	*
p70S6K Signaling	1.55	-0.45
B Cell Receptor Signaling	1.54	-0.82
Sumoylation Pathway	1.52	*
CTLA4 Signaling in Cytotoxic T Lymphocytes	1.46	*
Role of JAK1, JAK2 and TYK2 in Interferon Signaling	1.46	*
Glycolysis I	1.46	*
Prostate Cancer Signaling	1.45	*

Role of JAK family kinases in IL-6-type Cytokine Signaling	1.43	*
Gluconeogenesis I	1.43	*
Regulation of Cellular Mechanics by Calpain Protease	1.41	*
Actin Nucleation by ARP-WASP Complex	1.39	*
Thrombin Signaling	1.37	-1.34
VEGF Signaling	1.36	*
Protein Kinase A Signaling	1.33	2.12
Methylmalonyl Pathway	1.32	*
NAD Biosynthesis III	1.32	*
N-acetylglucosamine Degradation II	1.32	*
Chronic Myeloid Leukemia Signaling	1.31	*
Regulation of eIF4 and p70S6K Signaling	1.31	*

*The Z-score for the activation/suppression status could not be predicted.

Supplemental Table VII. Unique Differentially Expressed Genes in the Cancer-Stroke Group. See table in separate file.

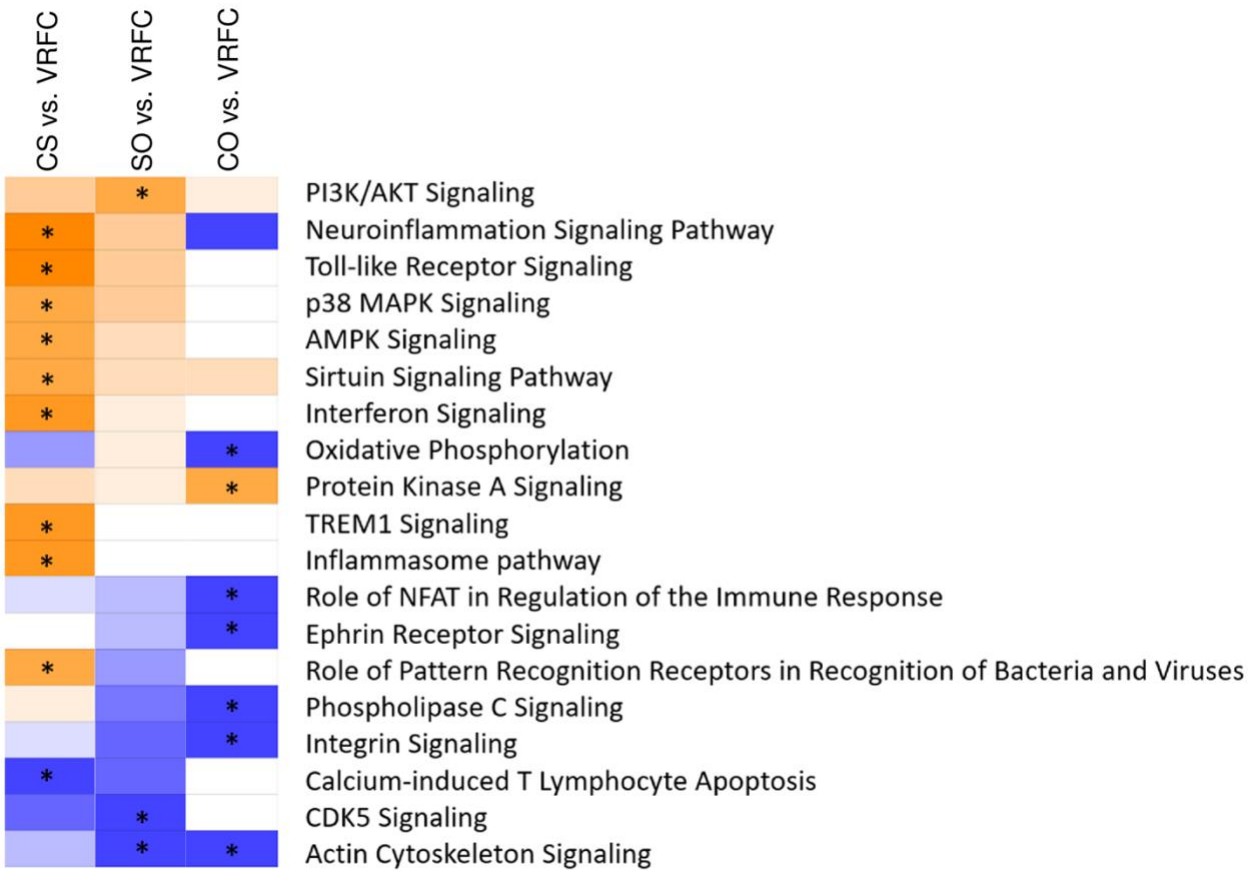
Supplemental Table VIII. Unique Differentially Expressed Genes in the Stroke-Only Group. See table in separate file.

Supplemental Table IX. Unique Differentially Expressed Genes in the Cancer-Only Group. See table in separate file.

Supplemental Table X. Differentially Expressed Genes between the Cancer-Stroke and Stroke-Only Groups. See table in separate file.

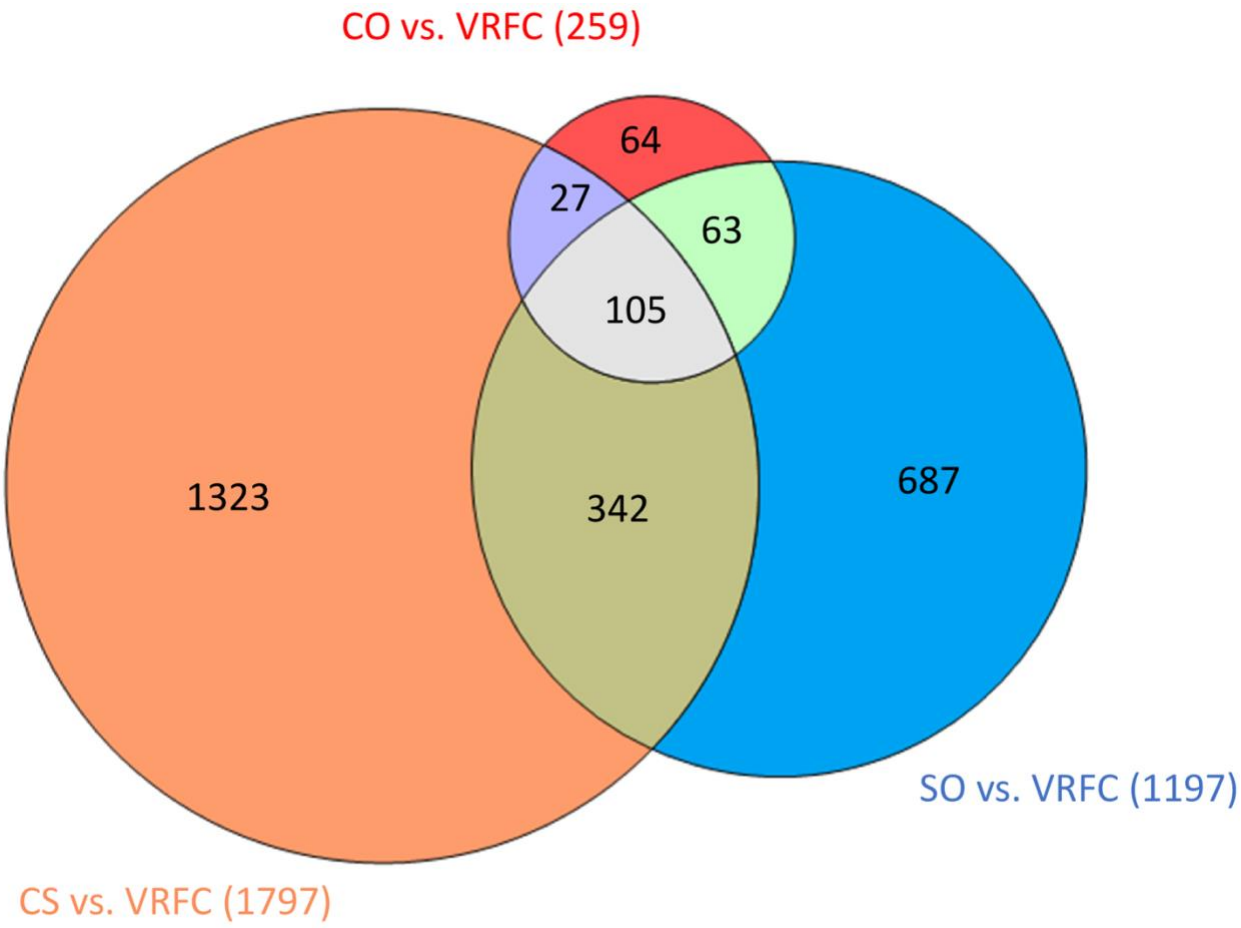
Supplemental Table XI. Differentially Expressed Genes between the Cancer-Stroke and Stroke-Only Groups after Excluding Differentially Expressed Genes between the Cancer-Only and Vascular Risk Factor Groups. See table in separate file.

Supplemental Figure IA



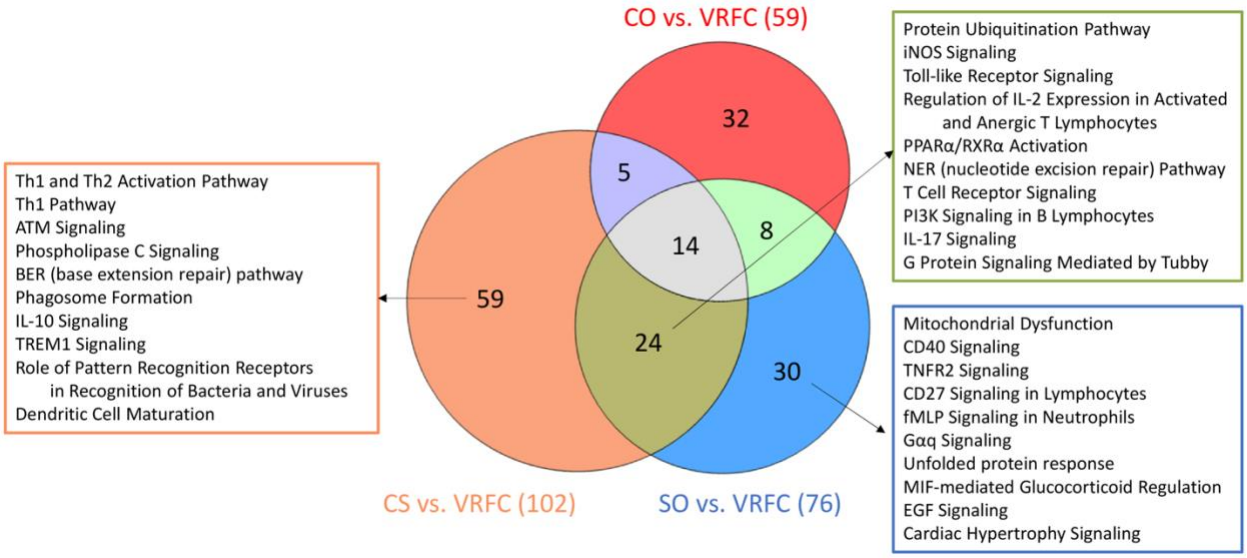
Legend. Overrepresented pathways in the cancer-stroke (CS), stroke-only (SO), and cancer-only (CO) groups compared to the vascular risk factor control (VRFC) group. Only overrepresented pathways for which at least one group had significant activation or suppression are presented. Orange denotes activation; blue denotes suppression; white denotes Z=0 or no genes populating the pathway. Hues depict the magnitude of the Z-score activation/suppression pattern. Asterisk denotes significant activation (Z>2) or suppression (Z<-2).

Supplemental Figure IB



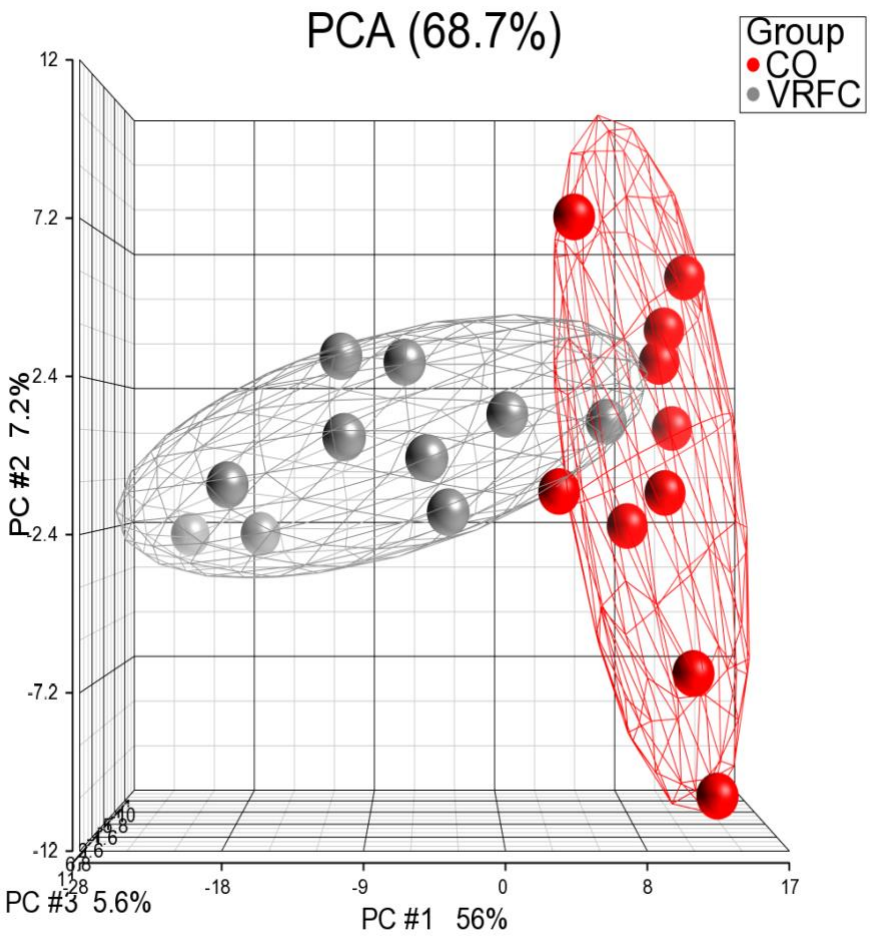
Legend. Overlap between the differentially expressed genes. The cancer-stroke group is abbreviated as CS, the cancer-only group is abbreviated as CO, the stroke-only group is abbreviated as SO, and the vascular risk factor control group is abbreviated as VRFC.

Supplemental Figure IC



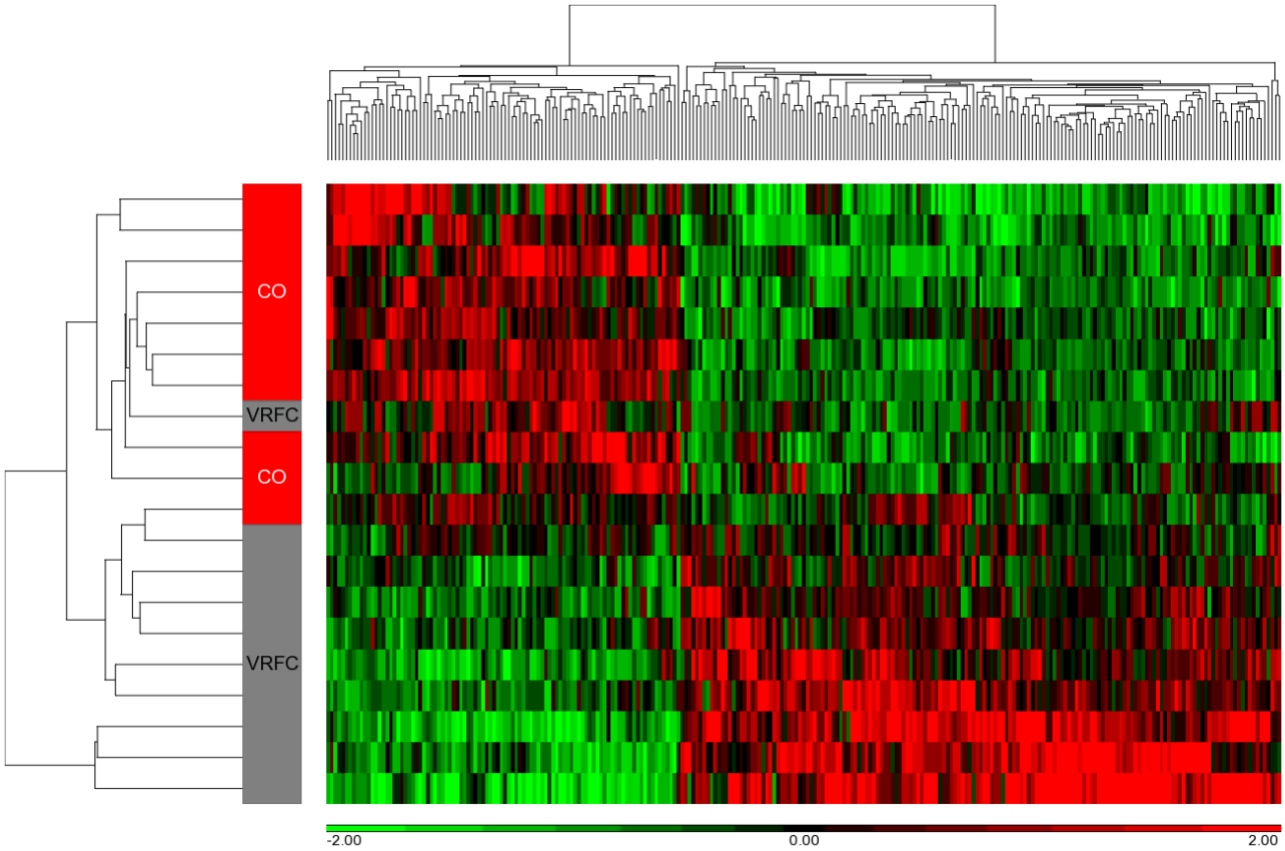
Legend. Overlap between the significantly overrepresented pathways. The top 10 overrepresented signaling pathways unique and common to the cancer-stroke (CS) and stroke-only (SO) groups are listed. CO is the abbreviation for the cancer-only group and VRFC is the abbreviation for the vascular risk factor control group.

Supplemental Figure IIA



Legend. Principal component analysis of the 259 genes differentially expressed between the cancer-only group and the vascular risk factor control group. The cancer-only group, abbreviated CO, is denoted by the red spheres; while the vascular risk factor control group, abbreviated VRFC, is denoted by the gray spheres.

Supplemental Figure IIB



Legend. Unsupervised hierarchical clustering of the 259 genes differentially expressed between the cancer-only group and the vascular risk factor control group. The cancer-only group is abbreviated as CO, while the vascular risk factor control group is abbreviated as VRFC. Red denotes high expressed genes while green denotes low expressed genes.

Supplemental References

1. Lee AY, Levine MN, Baker RI, Bowden C, Kakkar AK, Prins M, et al. Low-molecular-weight heparin versus a coumarin for the prevention of recurrent venous thromboembolism in patients with cancer. *New Engl J Med*. 2003;349:146-153
2. IDSA Practice Guidelines. Infectious Disease Society of America. https://www.idsociety.org/practice-guideline/practice-guidelines/#/date_na_dt/DESC/0/+/. Accessed March 23, 2019.
3. Stamova B, Ander BP, Jickling G, Hamade F, Durocher M, Zhan X, et al. The intracerebral hemorrhage blood transcriptome in humans differs from the ischemic stroke and vascular risk factor control blood transcriptomes. *J Cereb Blood Flow Metab*. 2018:271678X18769513
4. Adams HP, Jr., Bendixen BH, Kappelle LJ, Biller J, Love BB, Gordon DL, et al. Classification of subtype of acute ischemic stroke. Definitions for use in a multicenter clinical trial. Toast. Trial of org 10172 in acute stroke treatment. *Stroke*. 1993;24:35-41
5. Yatsenko SA, Mittal P, Wood-Trageser MA, Jones MW, Surti U, Edwards RP, et al. Highly heterogeneous genomic landscape of uterine leiomyomas by whole exome sequencing and genome-wide arrays. *Fertil Steril*. 2017;107:457-466 e459
6. Varga G, Mikala G, Kiss KP, Kosoczki E, Szabo E, Meggyesi N, et al. Proteasome subunit beta type 1 p11a polymorphism is a new prognostic marker in multiple myeloma. *Clin Lymphoma Myeloma Leuk*. 2017;17:734-742
7. Kawai K, Uemura M, Munakata K, Takahashi H, Haraguchi N, Nishimura J, et al. Fructose-bisphosphate aldolase a is a key regulator of hypoxic adaptation in colorectal cancer cells and involved in treatment resistance and poor prognosis. *Int J Oncol*. 2017;50:525-534