Some common pathways perturbed in septic shock and cancer

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Abstract

Sepsis and cancer are both leading causes of death, and occurrence of any one, cancer or sepsis, increases the likelihood of the other. While cancer patients are susceptible to sepsis, survivors of sepsis are also susceptible to develop certain cancers. This mutual dependence for susceptibility suggests shared biology between the two disease types. Earlier work in our laboratory had revealed cancer-related pathway to be up-regulated in Septic Shock (SS), an advanced stage of sepsis. In the present study, we performed comprehensive genome-scale analysis of published human transcriptome datasets from septic shock and 17 cancer types. We identified a total of 66 pathways perturbed in both septic shock and cancer. Based on enrichment scores of these pathways, some cancers were observed to be similar to sepsis (Sepsis Like Cancers - SLC group) but not others. SLC group consisted of malignancies of the gastrointestinal tract (liver, oesophagus, stomach, head and neck and biliary system) which are associated with infection. SLC group showed similar direction of change in gene expression as in septic shock while the malignancies of kidney, lung and uterus showed disagreement. The SLC group shared a large number of up-regulated pathways with SS, possibly representing the theme of dysregulated host response to infection. Notably, Galactose metabolism and p53 signalling pathways were significantly up-regulated in these cancer types along with septic shock group. This study highlights the complexity of the cancer transcriptome when viewed through the lens of septic shock.

Introduction

Sepsis is a potentially life-threatening complication caused by dysregulated host response to infection, often leading to organ failure and death. Estimated global burden of sepsis is more than 30 million people every year with 5 million deaths [1]. Newborns and children are more vulnerable with estimated incidence of 3 and 1.2 million, respectively [2]. Septic shock is the advanced stage of sepsis with metabolic dysregulation and uncontrolled hypotension. It is evident now from several epidemiological studies that sepsis and

cancer are associated. Liu et al.(2018) [3] conducted an association study between sepsis and ensuing risk of cancer in elderly adult population of United States, and found that the former is significantly associated with increased risk for many cancers including chronic myeloid leukemia (CML), myelodysplastic syndrome, acute myeloid leukemia (AML), cancers of cervix, liver, lung, rectum, colon. Another association study revealed 2.5 fold increased risk of sepsis in survivors of cancer in community-dwelling adults (the risk is increased up to 10 times in hospitalized cancer patients) [4]. Co-occurrence of cancer with sepsis is associated with higher mortality than sepsis alone without cancer [5]. On the other hand, sepsis is a common cause of death in critically ill patients with cancer, with high ICU and hospital mortality [6], [7]. Analysis of data from the past two decades revealed increasing trend in the incidence of postoperative (related to major cancer surgeries) sepsis [8]. There are previous reports on molecular changes in sepsis and cancer. Bergenfelz et al. (2012) [9] reported that Wnt5a induces immunosuppressive phenotype of macrophages in sepsis and breast cancer patients. HMGB1, a key late inflammatory mediator of systemic inflammatory response syndrome associated with bacterial sepsis, is also implicated in tumorigenesis and disease progression [10]. Muscle wasting, observed in patients with cancer, severe injury and sepsis, is associated with increased expression of several genes, particularly transcription factors and nuclear cofactors, regulating different proteolytic pathways [11]. CD11b/CD18 expression in monocytes has been proposed as a biomarker of infection in cancer patients [12]. These studies focused on the gene as the functional unit of analysis. On the other hand, a gene set or pathway represents coordinated molecular activity and captures higher-order functional unit in a tissue or cell. Pathway-level analysis allows detection of a cumulative signal that is not accessible at the gene-level. We did not find any report in literature about pathway-level comparative analysis between sepsis and cancer. In the present study, we have performed unbiased analysis of SS and cancer datasets to discover shared patterns of pathway perturbation between cancer and SS.

Materials and methods

Gene expression data for 17 different human cancers were retrieved from TCGA database (`https://portal.gdc.cancer.gov/`) on July 5, 2018. For each tissue, TCGA project code was provided in the search field and RNA-seq data for paired samples (case-control) were downloaded. Likewise, transcriptome data of human septic shock (SS) were retrieved from Gene Expression Omnibus (GEO) database on April 10, 2019. Retrieved expression data were log2-transformed. Gene Set Enrichment Analysis (GSEA) on expression data of both cancer and SS was performed as published earlier [13]. Briefly, enrichment score (ES) for each pathway was calculated by dividing the sum of the gene-level log-fold changes with the square root of the pathway size. This provided a pathway-level score which was highly positive for up-regulated pathways and highly negative for the down-regulated pathways. Significance of the observed score (ES) was estimated in the following manner. By scrambling the case/control labels, new data were simulated and ES recalculated for each simulation. When repeated over

many (i.e., 10000) iterations, this generated the null distribution of the simulated ES for each pathway. Fraction of the ES null distribution that was more extreme than the observed ES was estimated to be the p-value associated with the pathway [14]. Selection of the transcriptome data sets and analysis workflow leading to the final list of significant pathways are described in Figure 1. The study characteristics of the data sets are listed in Table 1. We performed network analysis using each pathway as node and shared genes between pathways as their connecting edges. Firstly, a background network was constructed by including all the pathways (KEGG [15]) with overlapping gene memberships, i.e., for a pathway to be included, it must share at least 5% of the total number of genes with another pathway. In this network, each pathway was considered a node, and the edge between two nodes suggested overlap between the two pathways. The nodes in the network were coloured according to the disease-group in which the node (pathway) was perturbed: SLC (sepsis-like cancer), CA-only (uniquely cancer) or both. All programming was done in the R programming language (R core team 2013) [16]. The code is available on request.

Results

Out of 290 KEGG pathways, 90 were significantly perturbed in SS group ($p < 0.01$). Hierarchical clustering on combined cancer and sepsis data sets for these 90 pathways revealed two groups of cancers: which segregated with sepsis (sepsis-like cancer, or SLC) and which did not segregate with SS (uniquely cancer, or CA) (Figure 2). Out of 17 cancer types, 11 uniquely cancer studies were grouped (CA group) under the first clade of the heatmap. The CA group comprised of BLCA, BRCA, COAD, KICH, KIRP, LUAD, LUSC, PRAD, READ, THCA and UCEC. Second clade was formed with other cancer studies (LIHC, CHOL, KIRC, HNSC, ESCA and STAD, together termed the SLC group) and 6 SS studies. Of the 90 pathways, we further selected those pathways which were significantly perturbed in at least 80% of one or both of the cancer groups (SLC and CA) leading to retention of 66 pathways (Figure 3) which were subjected to further analysis. Many of the 66 SS-associated pathways were significantly perturbed in cancer including KICH (66), CHOL and LIHC (64 each), HNSC (63), STAD (60), ESCA (57), LUSC (51), KIRC (50). While the SLC group showed more than 75% of the selected pathways to be associated with SS, many of the CA group cancers showed less association with these pathways. For example, except KICH (66) and LUSC (51), most CA group cancers had either low number of associated pathways - THCA (21), PRAD (12), READ (10) – or showed intermediate association, such as BRCA (49), UCEC (45), COAD (39), KIRP (33), LUAD (33), BLCA (30) and THCA (21). In general, the pathways were up-regulated in the SLC group (in the same direction as SS, with more intensity) and down-regulated (in a direction opposite to SS) in the CA group. Two cancer associated KEGG pathways were found to be differentially perturbed in both cancer and SS such as *Pathways in cancer* (Supplementary Figure S1) and Transcriptional mis-regulation in cancer (Supplementary Figure S2).

Interestingly, all three down-regulated pathways (Ribosome biogenesis in eukaryotes,

Antigen processing and presentation, and Primary immunodeficiency) in SS group were up-regulated in SLC group. In order to ascertain relative importance of each pathway, we performed network analysis. We considered each pathway as a node and its connection with the next node based on sharing of genes between them. If two pathways shared at least 5% of genes common among themselves, then they were considered connected. In this way, we generated a network of KEGG pathways with 244 nodes (each node being a KEGG pathway) and 5304 edges. The overall property of the network was assessed by looking at the degree distribution of the nodes (Figure 4). As expected of all biological networks, there are many nodes with few edges between them and some nodes with many edges (Figure 5). By colouring the three principal groups of pathways with three different colours, it is revealed that the SLC-only nodes (coloured red) are located in the core of the network, while the other nodes are more peripheral. Of the pathways detected as significant in this analysis, some stand out for their biological and clinical relevance. These are described below.

Lysosome: Lysosomal pathway was found to be differentially perturbed in both SS and SLC (Supplementary Figure S3). Lysosomes are membrane-bound catabolic organelles that maintain cellular homeostasis. Marked changes in composition and function of lysosomes can be observed in disease conditions. Sepsis and cancer induced changes in lysosomes are pronounced and also share commonality between them. Muscle wasting is one of the most common phenomenon in critically ill patients with sepsis. Apart from proteasome system, lysosome pathway also plays important role in protein degradation [17] and degradation of autophagosomes generated after the onset of sepsis, has been implicated in cancer cachexia leading to muscle wasting in cancer [18]. Interestingly cancer cells are known to have increased biogenesis of lysosomes with different membrane composition, enhanced expression, activity and secretion of lysosomal enzymes [19]. Besides, Lysosomes are now considered as hub of metabolic signaling [20]. In our study, all members of SLC (except KIRC with non-significant positive ES) and SS groups have positive ES along with the UCEC, KIRP, THCA, BLCA and BRCA of the CA group (Figure 3). Ma et al. 2015 [21], reported up-regulation of lysosome pathway in SS. Upon investigation of the pathway in the network of pathways it was revealed that lysosome pathway has the second highest (after Glycolysis/Gluconeogenesis pathway: 1599.07) betweenness value of 680.2, showing its hub-like nature in the network.

Leukocyte transendothelial migration: Leukocyte transendothelial migration pathway was found to be differentially perturbed in both SS and SLC (Supplementary Figure S4). Migration of leukocytes from the blood into sites of tissue injury and infection, across the vascular endothelium, is a fundamental immune response to eliminate inflammatory trigger and help in tissue repair. Leukocyte transendothelial migration pathway is significantly up-regulated in SS and SLC groups as well as THCA of CA (Figure 3). However, most of cancers of CA group including BRCA, COAD, KICH, KIRP, LUAD, LUSC, PRAD and UCEC showed significant down-regulation of this pathway. Other pathways related to infection and inflammation, i.e., *Toll like receptor pathway* (Supplementary Figure S5), *TNF signaling pathway* (Supplementary Figure S6) and *RIG-I-like signalling pathway* (Supplementary Figure S7), *Bacterial invasion of*

Epithelial cell (Supplementary Figure S8), *Helicobacter pylori infection* (Supplementary Figure S9), or movement of cells, i.e., *Regulation of actin cytoskeleton (Supplementary Figure S10),* were also up-regulated in SLC and SS.

Galactose metabolism: Galactose metabolism pathway was found to be differentially perturbed in both SS and SLC (Supplementary Figure S11). In humans, d-galactose is generated after breakage of lactose or catabolic event of glycoproteins and glycolipids by glycoside hydrolase enzymes (Alpha-galactosidase (α-GAL, also known as α-GAL A; E.C. 3.2.1.22)). Finally, d-Galactose is converted into many intermediate metabolites and may enter any of the carbohydrate metabolism pathways (e.g. Fructose and mannose metabolism, glycolysis, pentose phosphate pathway). However, any imbalance in galactose metabolism that increases levels of galactose or galactose 1-phosphate may lead to galactosemia. Galactosemia has been implicated in neonatal sepsis [22], [23]. Further, high level of d-galactose is reported in cases of sepsis [24]. Galactose metabolism pathway is also up-regulated in SLC (except STAD) and SS groups. Likewise, we found this pathway up-regulated in BLCA, KIRP, LUAD, LUSC and UCEC while down-regulated in COAD, KICH, PRAD and READ in CA group. UDP-galactose (mainly derived from d-galactose via Leloir pathway) provide galactosyl entity for the biosynthesis of glycolipids and glycoproteins that play important role in receptor-mediated signalling, cell-cell recognition and metastasis in cancer cells [25].

Bladder cancer: Bladder cancer pathway was found to be differentially perturbed in both SS and SLC (Supplementary Figure S12). Notably, bladder cancer pathway is up-regulated in all the diseases of SLC and SS groups and many diseases of CA group including BLCA, BRCA, COAD, LUAD, LUSC, READ, THCA and UCEC as well.

p53 signaling pathway: p53 signaling pathway was found to be differentially perturbed in both SS and SLC (Supplementary Figure S13). Stress signals (i.e. oxidative stress, DNA damage, oncogene activation etc.) induce p53 activation leading to enhanced transcription of p53-regulated genes, resulting in DNA damage repair, cell cycle arrest, cellular senescence or apoptosis. Like bladder cancer pathway, p53 signaling pathway is also significantly up-regulated in all disease types of SLC, SS and CA groups (except KICH and PRAD).

Ribosome biogenesis in eukaryotes: Ribosome biogenesis in eukaryotes was found to be differentially perturbed in both SS and SLC (Supplementary Figure S14). Ribosomes are sites of protein synthesis in the cell, and their biogenesis is vital for the cell growth and division. Interestingly, we found down-regulation of ribosome biogenesis pathway in all SS datasets, but up-regulation in CA and SLC groups including BLCA, BRCA, COAD, KIRP, LUAD, LUSC, PRAD, READ and UCEC.

Discussion

Septic shock is a lethal condition with profound genome-scale change in expression. Earlier work in our laboratory had revealed cancer-associated pathways to be associated with septic shock [13], providing motivation for comparison of the transcriptomes between SS and cancer. By comprehensive transcriptome analysis of human septic

shock at the pathway level, we sought to capture the higher-order signal in the two clinical entities (sepsis and cancer). We identified 90 pathways that were significantly perturbed and mostly up-regulated in SS. Hierarchical clustering of perturbation signals clearly segregated the cancer studies into two groups: those perturbed in the same direction as SS, i.e., up-regulated (called SLC group) and those that were perturbed in the reverse direction (called CA group). The CA group shows general down-regulation (with lesser significance) in most of the pathways compared to SLC. On the other hand, the SLC group showed similar (if somewhat accentuated) pathway gene expression profile as SS. Five out of six of the SLC group cancers belonged to that of the gastrointestinal system (head and neck, esophagus, stomach, liver and biliary system). These cancers are often associated with infection (i.e., human papilloma virus in "head and neck", *H. pylori* in stomach, hepatitis viruses in liver). This is of biological significance as SS is caused by abnormal host response to infection leading to systemic inflammation and organ failure, and inflammation is also a common theme in many cancers. The similar pathway signature between SS and SLC suggests common biological processes involving dysregulated host response to infection at different stages of disease pathogenesis. This has implications for management of different cancers which needs further investigation.

In conclusion, comparative transcriptomics of septic shock and cancer has revealed strong similarity of a groups of cancers (called SLC group here) with septic shock. These malignancies include many of the gastrointestinal tract that are often associated with infection. Robust up-regulation of a large number of pathways in septic shock is accentuated in the SLC cancers, which suggests a common biological theme running through these two distinct clinical entities. This is the first attempt to view the cancer transcriptome through the lens of septic shock. It is hoped that further work shall translate this result to actionable knowledge for clinical management of both cancer and septic shock.

Author contributions

SKM conceptualized the study and contributed to Funding Acquisition, Project Administration, Resources, Supervision. HT contributed to Data Curation. SM contributed to Data Curation, Software, Validation, Visualization. All authors contributed to Formal Analysis, Investigation, Methodology, Manuscript writing and approved of the final manuscript.

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References

1. Fleischmann C, Scherag A, Adhikari NK, Hartog CS, Tsaganos T, Schlattmann P, et al. Assessment of global incidence and mortality of hospital-treated sepsis. Current estimates and limitations. American journal of respiratory and critical care medicine. 2016;193(3):259–272.

2. Fleischmann-Struzek C, Goldfarb DM, Schlattmann P, Schlapbach LJ, Reinhart K, Kissoon N. The global burden of paediatric and neonatal sepsis: a systematic review. The Lancet Respiratory Medicine. 2018;6(3):223–230.

3. Liu Z, Mahale P, Engels EA. Sepsis and Risk of Cancer Among Elderly Adults in the United States. Clinical Infectious Diseases. 2018;68(5):717–724.

4. Moore JX, Akinyemiju T, Bartolucci A, Wang HE, Waterbor J, Griffin R. A prospective study of cancer survivors and risk of sepsis within the REGARDS cohort. Cancer epidemiology. 2018;55:30–38.

5. Dagher GA, El Khuri C, Chehadeh AAH, Chami A, Bachir R, Zebian D, et al. Are patients with cancer with sepsis and bacteraemia at a higher risk of mortality? A retrospective chart review of patients presenting to a tertiary care centre in Lebanon. BMJ open. 2017;7(3):e013502.

6. Torres VB, Azevedo LC, Silva UV, Caruso P, Torelly AP, Silva E, et al. Sepsis-associated outcomes in critically ill patients with malignancies. Annals of the American Thoracic Society. 2015;12(8):1185–1192.

7. Rosolem MM, Rabello LS, Lisboa T, Caruso P, Costa RT, Leal JV, et al. Critically ill patients with cancer and sepsis: clinical course and prognostic factors. Journal of critical care. 2012;27(3):301–307.

8. Sammon JD, Klett DE, Sood A, Olugbade Jr K, Schmid M, Kim SP, et al. Sepsis after major cancer surgery. journal of surgical research. 2015;193(2):788–794.

9. Bergenfelz C, Medrek C, Ekström E, Jirström K, Janols H, Wullt M, et al. Wnt5a induces a tolerogenic phenotype of macrophages in sepsis and breast cancer patients. The Journal of Immunology. 2012;188(11):5448–5458.

10. Diener KR, Al-Dasooqi N, Lousberg EL, Hayball JD. The multifunctional alarmin HMGB1 with roles in the pathophysiology of sepsis and cancer. Immunology and cell biology. 2013;91(7):443–450.

11. Aversa Z, Alamdari N, Hasselgren PO. Molecules modulating gene transcription during muscle wasting in cancer, sepsis, and other critical illness. Critical reviews in clinical laboratory sciences. 2011;48(2):71–86.

12. Ravetti CG, Moura AD, Teixeira AL, Pedroso ÊRP. Sepsis in cancer patients admitted in the ICU: epidemiology, pathophysiology, and biomarkers. Rev Med Minas Gerais. 2014;24(3):383–389.

13. Mukhopadhyay S, Thatoi PK, Pandey AD, Das BK, Ravindran B, Bhattacharjee S, et al. Transcriptomic meta-analysis reveals up-regulation of gene expression functional in osteoclast differentiation in human septic shock. PloS one. 2017;12(2):e0171689.

14. Oron AP, Jiang Z, Gentleman R. Gene set enrichment analysis using linear models and diagnostics. Bioinformatics. 2008;24(22):2586–2591.

15. Ogata H, Goto S, Sato K, Fujibuchi W, Bono H, Kanehisa M. KEGG: Kyoto encyclopedia of genes and genomes. Nucleic acids research. 1999;27(1):29–34.

16. Team R, et al. R: A language and environment for statistical computing. 2013;.

17. Klaude M, Mori M, Tjäder I, Gustafsson T, Wernerman J, Rooyackers O. Protein metabolism and gene expression in skeletal muscle of critically ill patients with sepsis. Clinical science. 2012;122(3):133–142.

18. Penna F, Costamagna D, Pin F, Camperi A, Fanzani A, Chiarpotto EM, et al. Autophagic degradation contributes to muscle wasting in cancer cachexia. The American journal of pathology. 2013;182(4):1367–1378.

19. Kallunki T, Olsen O, Jäättelä M. Cancer-associated lysosomal changes: friends or foes? Oncogene. 2013;32(16):1995.

20. Lamming DW, Bar-Peled L. Lysosome: The metabolic signaling hub. Traffic. 2019;20(1):27–38.

21. Ma J, Chen C, Barth AS, Cheadle C, Guan X, Gao L. Lysosome and cytoskeleton pathways are robustly enriched in the blood of septic patients: a meta-analysis of transcriptomic data. Mediators of inflammation. 2015;2015.

22. Rathi N, Rathi A. Galactosemia presenting as recurrent sepsis. Journal of tropical pediatrics. 2011;57(6):487–489.

23. Verma S, Bharti B, Inusha P. Association of fungal sepsis and galactosemia. The Indian Journal of Pediatrics. 2010;77(6):695–696.

24. Wang Z, Wu B, Weng J, Sun F, Zeng M, Cherukury H, et al. Metabolic study in serum from patients with sepsis and severe sepsis. Int J Clin Exp Med. 2016;9(3):6551–6556.

25. Tang M, Etokidem E, Lai K. The Leloir pathway of galactose metabolism–a novel therapeutic target for hepatocellular carcinoma. Anticancer research. 2016;36(12):6265–6271.

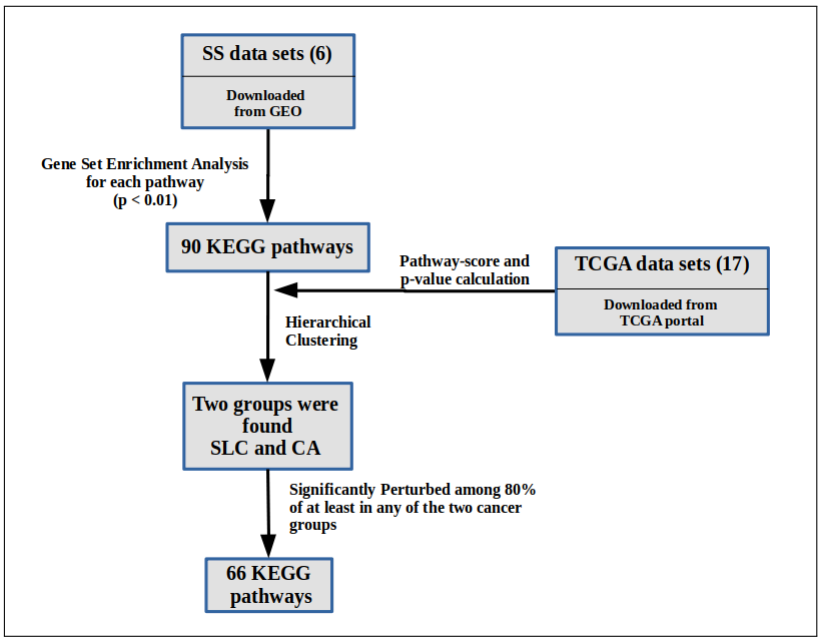


Fig 1. Selection of data and analysis workflow.

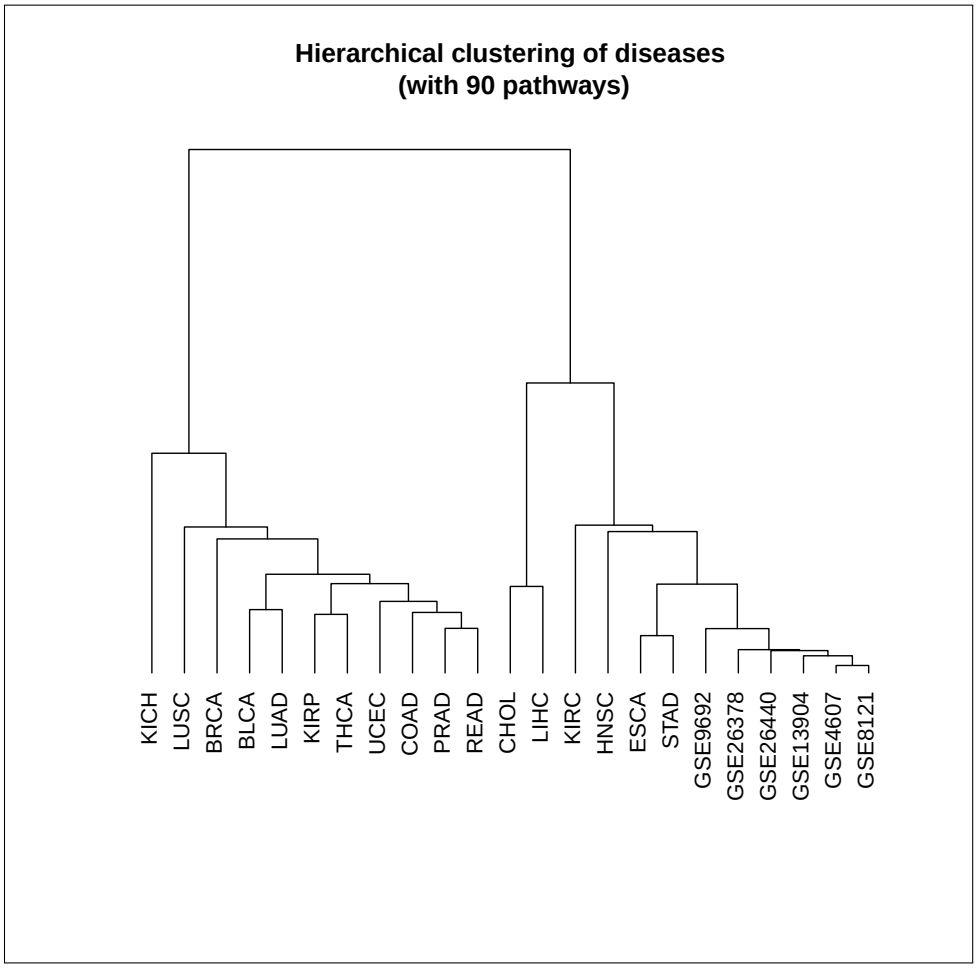


Fig 2. Hierarchical clustering of the 23 studies based on the pathway scores values for the 90 KEGG pathways.

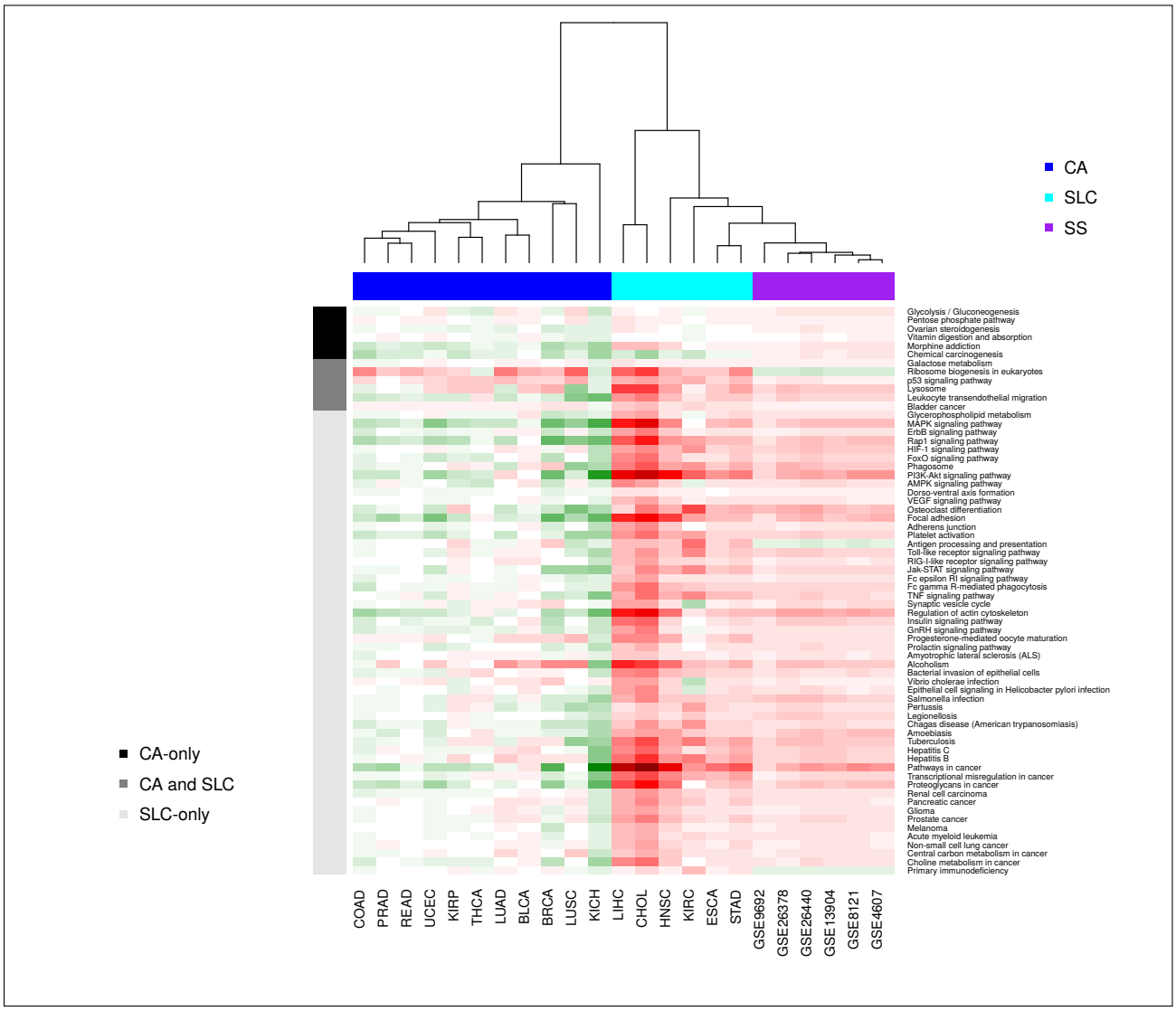


Fig 3. Pathway heat map for 66 selected KEGG pathways where each pathway is perturbed in at least 80% of the studies in one or both of the two groups of cancers. The column-side color bars suggest SS for purple, SLC for cyan and CA for blue. The row-side color bars suggest dark gray for CA-only pathways, gray for CA and SLC, light gray for SLC-only pathways.

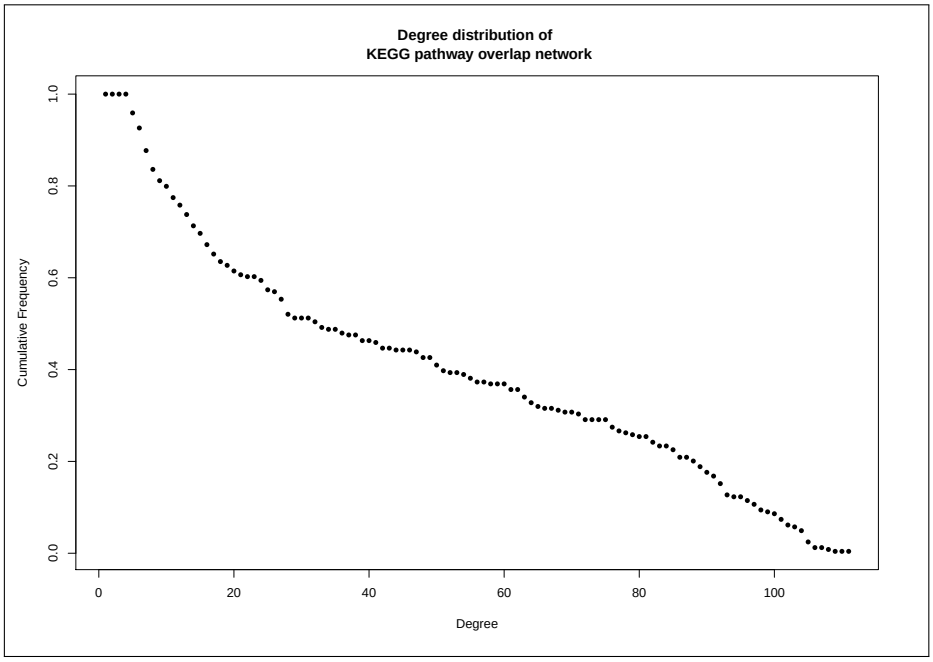


Fig 4. This is the degree distribution of the network generated by overlapping KEGG pathways. This shows that there are many nodes with very less connectivity but a few nodes with very high connectivity with other nodes.

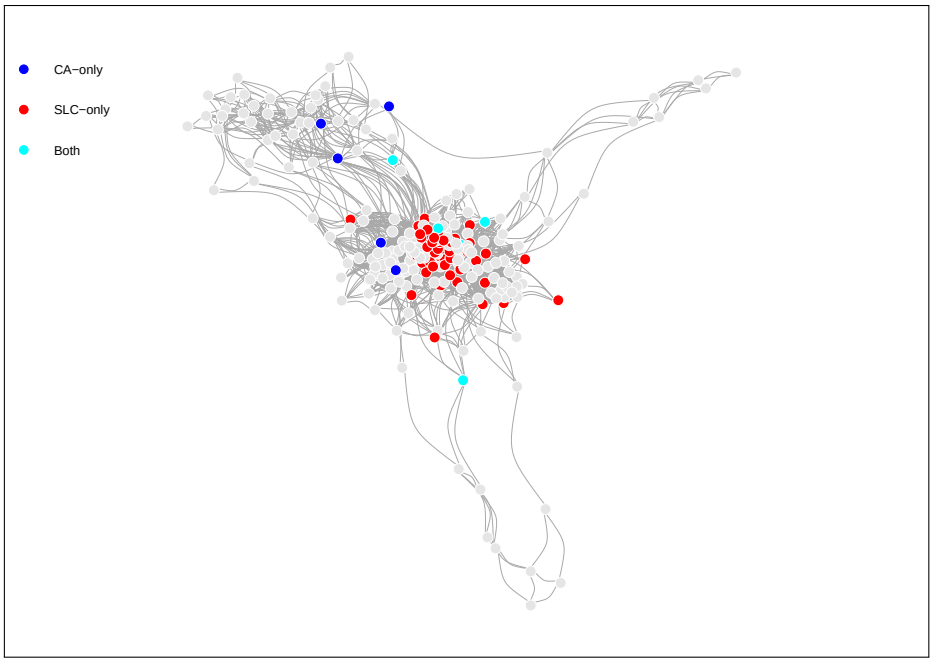
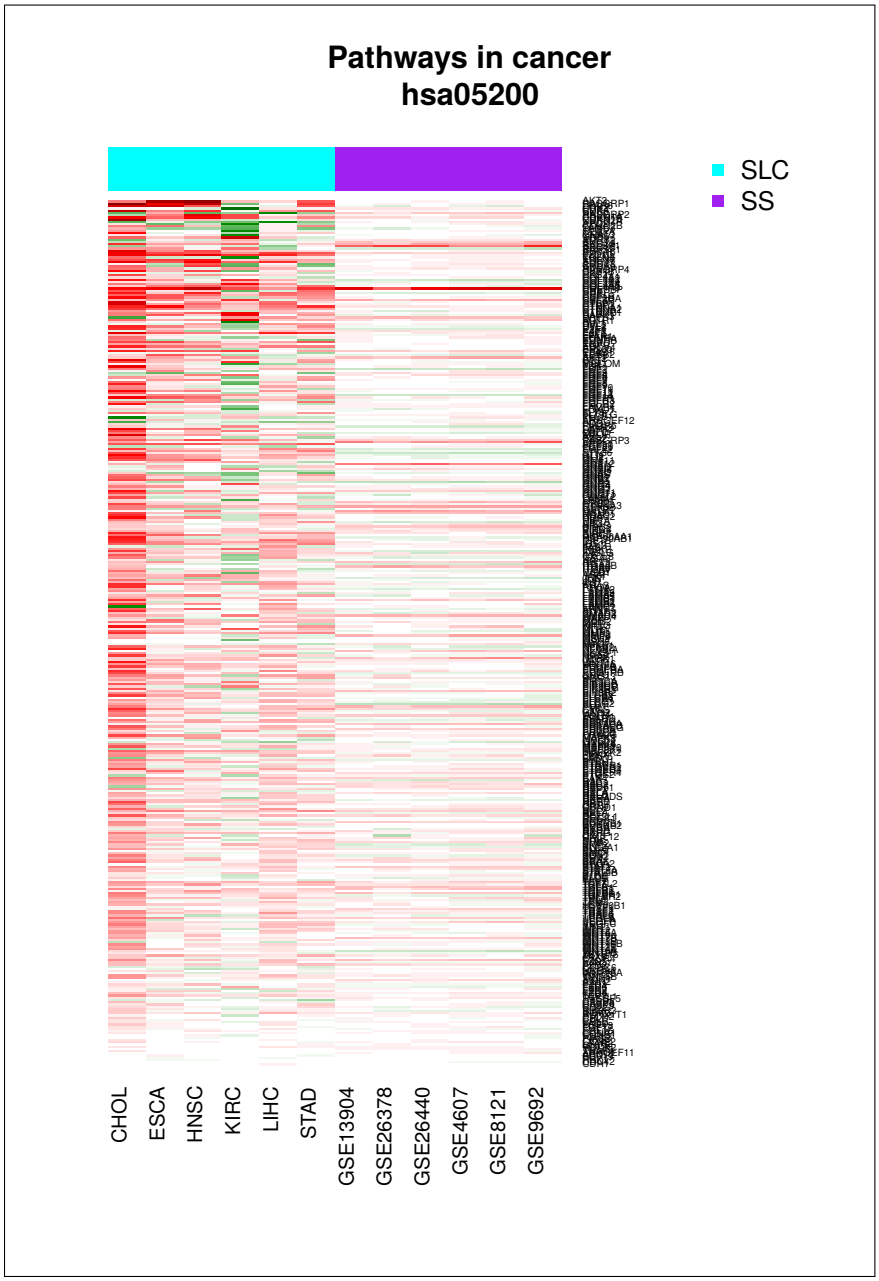


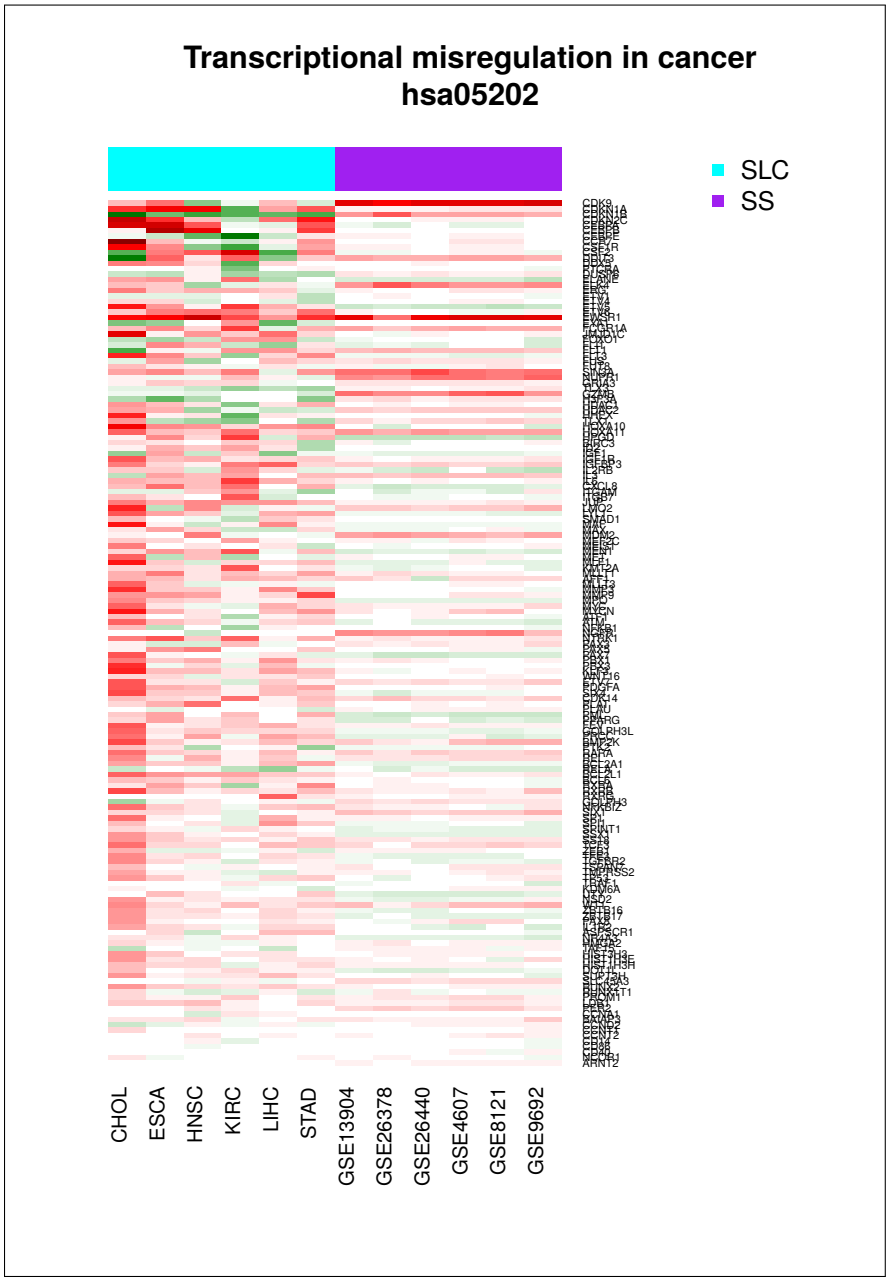
Fig 5. KEGG overlap network, with color-coding of the disease-specific pathways as listed on the top left. The SLC group of cancers are seen to be occupying a more central location of the entire KEGG network. This might suggest that key biological processes are shared between SS and SLC.

Study_Code	Study_Name	Data source	Disease	Tissue	Number of samples in the study	Paired control	Technology
BLCA	Bladder Urothelial Carcinoma	TCGA	Cancer	Bladder	38	Yes	RNA-seq (Illumina)
BRCA	Breast invasive carcinoma	TCGA	Cancer	Breast	220	Yes	RNA-seq (Illumina)
CHOL	Cholangiocarcinoma	TCGA	Cancer	Gallbladder, liver and parts of biliary tract	18	Yes	RNA-seq (Illumina)
COAD	Colon adenocarcinoma	TCGA	Cancer	Colon and rectosigmoid junction	82	Yes	RNA-seq (Illumina)
ESCA	Esophageal carcinoma	TCGA	Cancer	Esophagus	16	Yes	RNA-seq (Illumina)
HNSC	Head and Neck squamous cell carcinoma	TCGA	Cancer	Base of tongue, floor of mouth, gum, hypo- and oro-pharynx, larynx, etc.	86	Yes	RNA-seq (Illumina)
KICH	Kidney Chromophobe	TCGA	Cancer	Kidney	48	Yes	RNA-seq (Illumina)
KIRC	Kidney renal clear cell carcinoma	TCGA	Cancer	Kidney	144	Yes	RNA-seq (Illumina)
KIRP	Kidney renal papillary cell carcinoma	TCGA	Cancer	Kidney	64	Yes	RNA-seq (Illumina)
LIHC	Liver hepatocellular carcinoma	TCGA	Cancer	Liver and intrahepatic bile ducts	100	Yes	RNA-seq (Illumina)
LUAD	Lung adenocarcinoma	TCGA	Cancer	Bronchus and lung	114	Yes	RNA-seq (Illumina)
LUSC	Lung squamous cell carcinoma	TCGA	Cancer	Bronchus and lung	98	Yes	RNA-seq (Illumina)
PRAD	Prostate adenocarcinoma	TCGA	Cancer	Prostate gland	104	Yes	RNA-seq (Illumina)
READ	Rectum adenocarcinoma	TCGA	Cancer	Rectum rectosigmoid junction	20	Yes	RNA-seq (Illumina)
STAD	Stomach adenocarcinoma	TCGA	Cancer	Stomach	62	Yes	RNA-seq (Illumina)
THCA	Thyroid carcinoma	TCGA	Cancer	Thyroid gland	116	Yes	RNA-seq (Illumina)
UCEC	Uterine Corpus Endometrial Carcinoma	TCGA	Cancer	Corpus uteri	46	Yes	RNA-seq (Illumina)
GSE4607	Septic Shock	GEO	Septic Shock	Whole Blood	84	No	Microarray (Affymetrix HGU 133 Plus 2.0)
GSE8121	Septic Shock	GEO	Septic Shock	Whole Blood	75	No	Microarray (Affymetrix HGU 133 Plus 2.0)
GSE9692	Septic Shock	GEO	Septic Shock	Whole Blood	45	No	Microarray (Affymetrix HGU 133 Plus 2.0)
GSE13904	Septic Shock	GEO	Septic Shock	Whole Blood	124	No	Microarray (Affymetrix HGU 133 Plus 2.0)
GSE26378	Septic Shock	GEO	Septic Shock	Whole Blood	103	No	Microarray (Affymetrix HGU 133 Plus 2.0)
GSE26440	Septic Shock	GEO	Septic Shock	Whole Blood	130	No	Microarray (Affymetrix HGU 133 Plus 2.0)

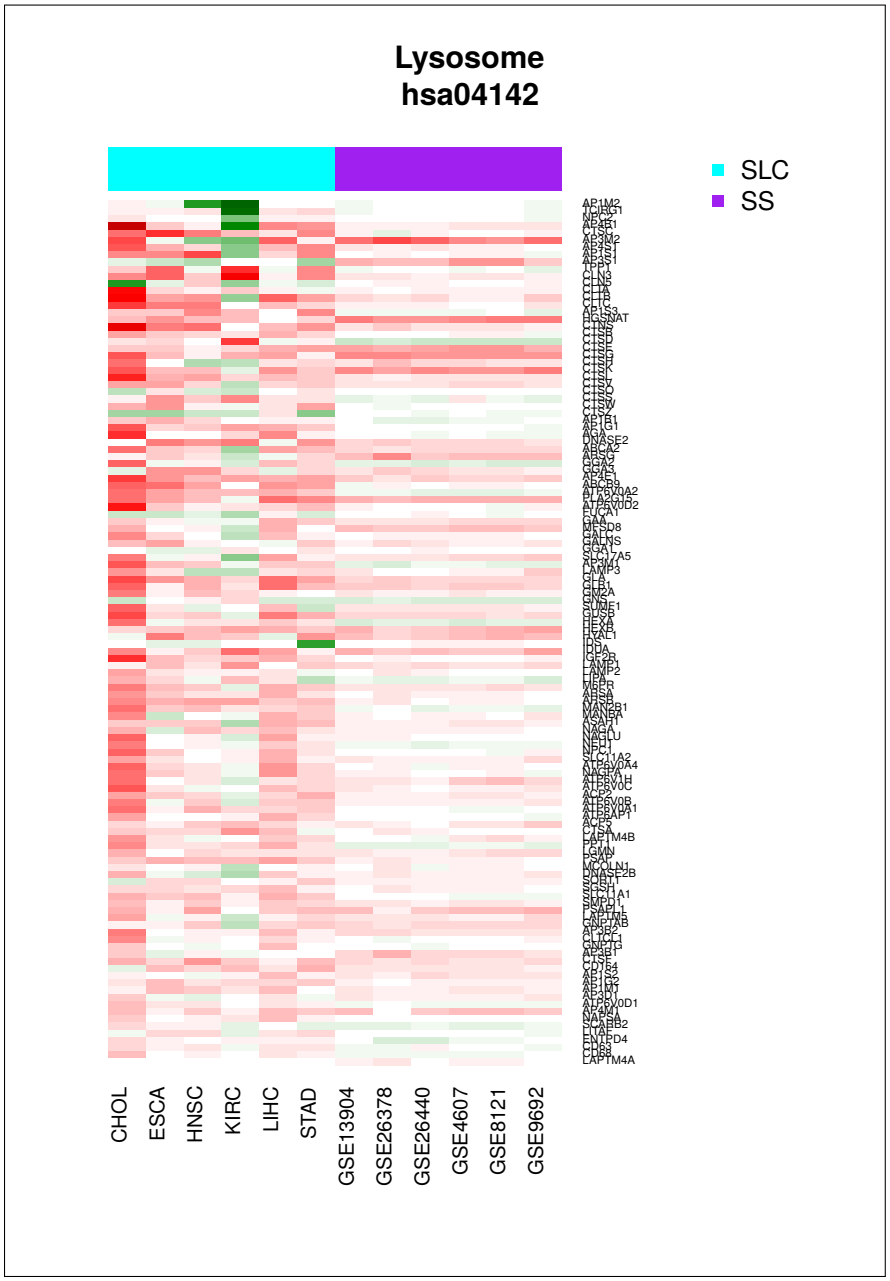
Table 1. Study characteristics of the 23 datasets selected for this study.



Supplementary Figure S1: [Pathways in cancer hsa05200] was observed to be up-regulated in both SS and SLC.



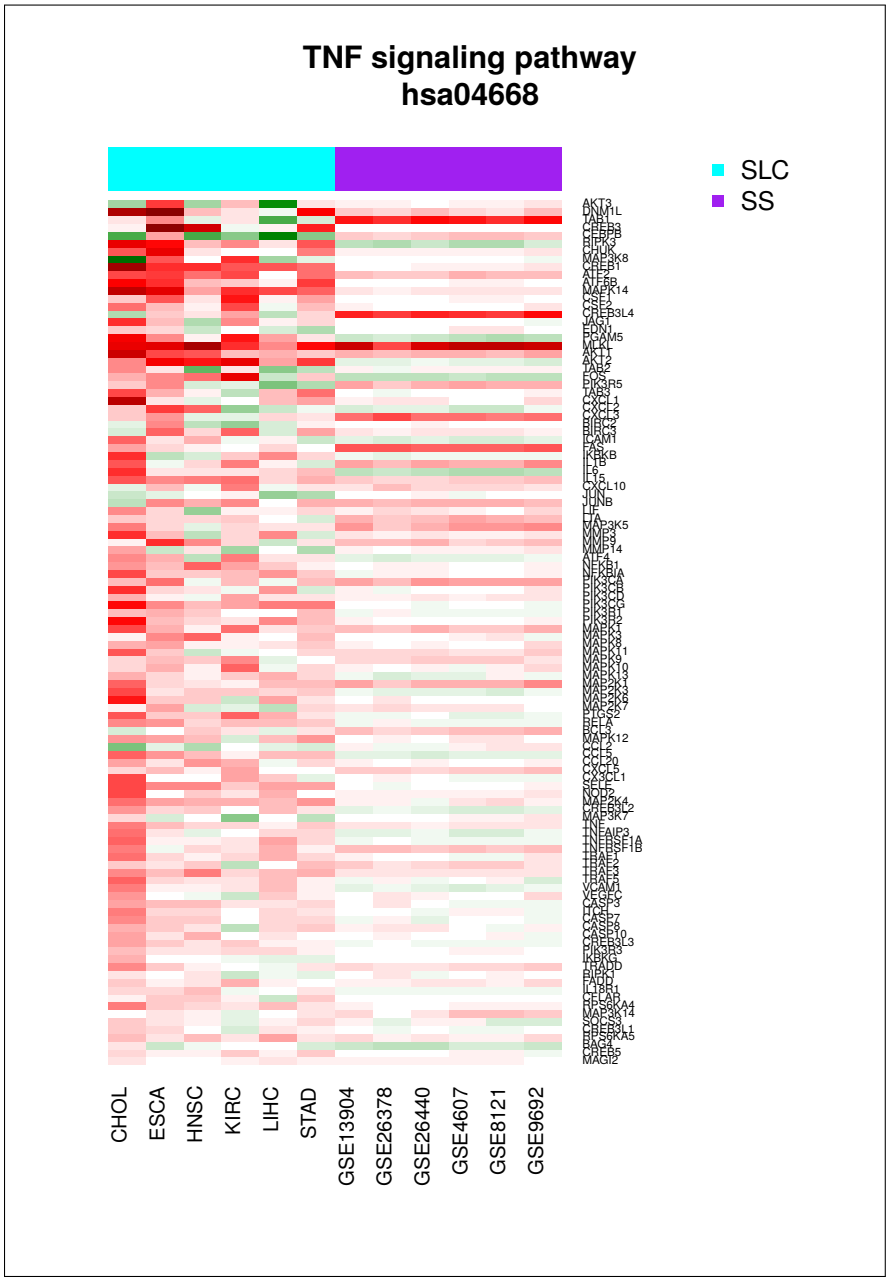
Supplementary Figure S2: [Transcriptional misregulation in cancer hsa05202] was observed to be up-regulated in both SS and SLC.



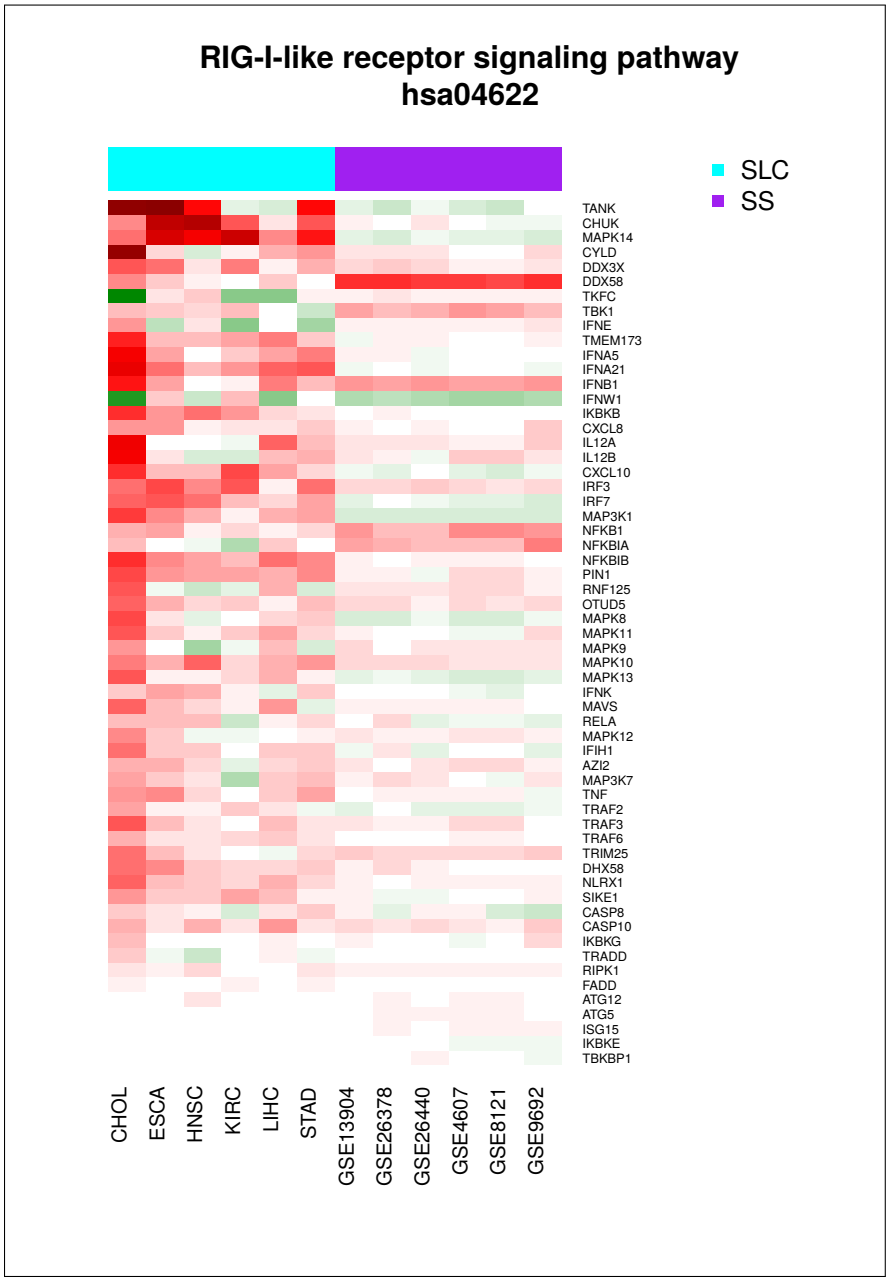
Supplementary Figure S3: [Lysosome pathway hsa04142] was observed to be up-regulated in both SS and SLC.

Supplementary Figure S4: [Leukocyte transendothelial migration hsa04670] was observed to be up-regulated in both SS and SLC.

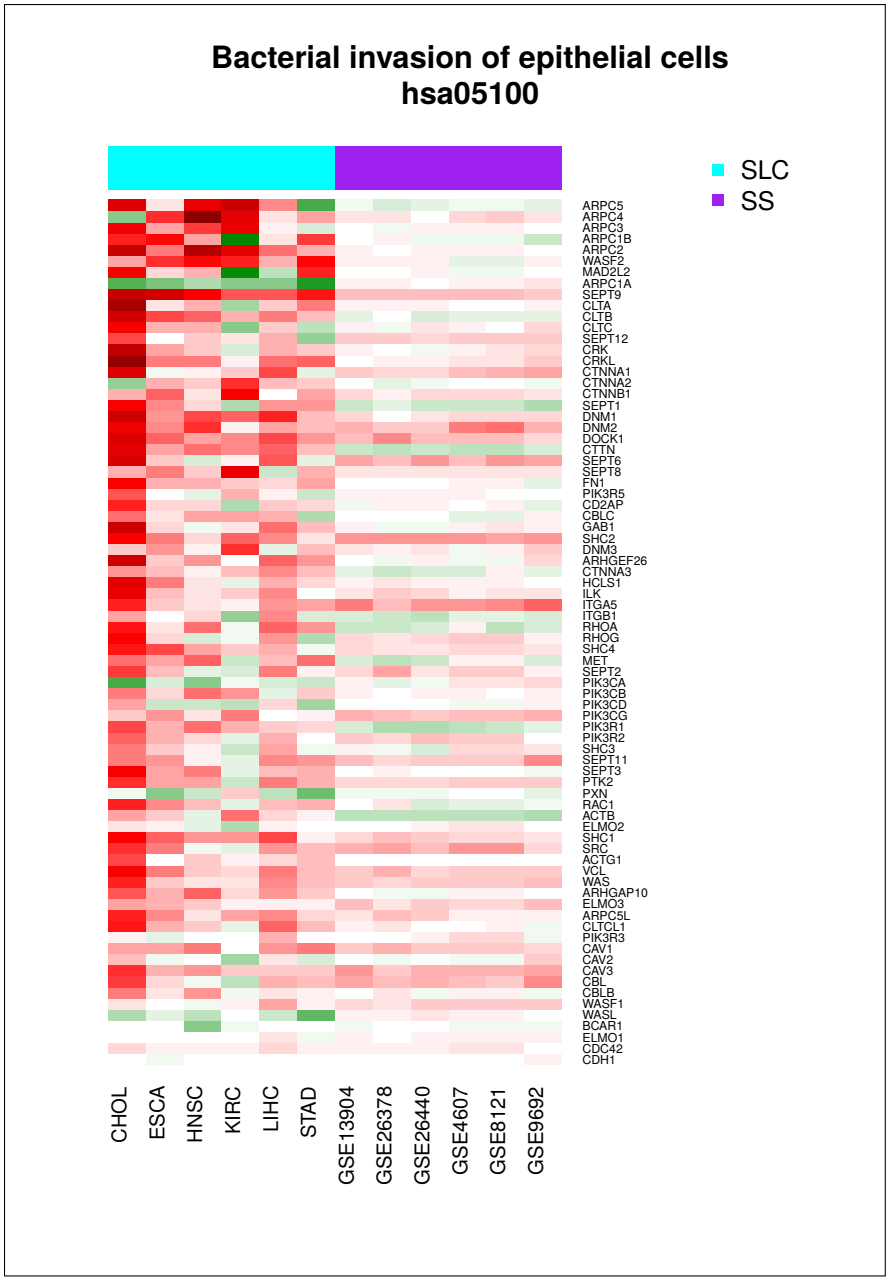
Supplementary Figure S5: [Toll like receptor pathway hsa04620] was observed to be up-regulated in both SS and SLC.



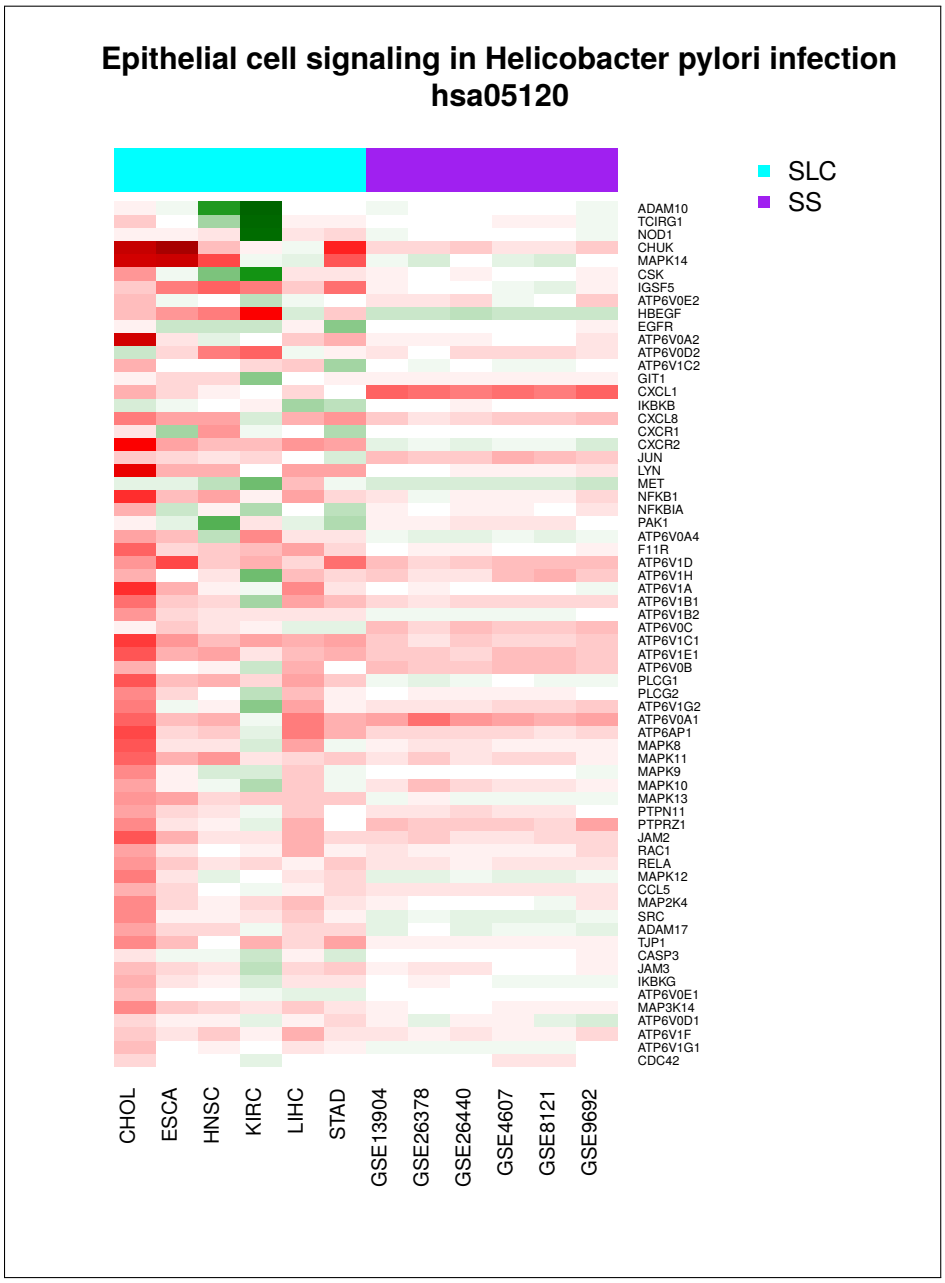
Supplementary Figure S6: [TNF signaling pathway hsa04668] was observed to be up-regulated in SS and SLC.



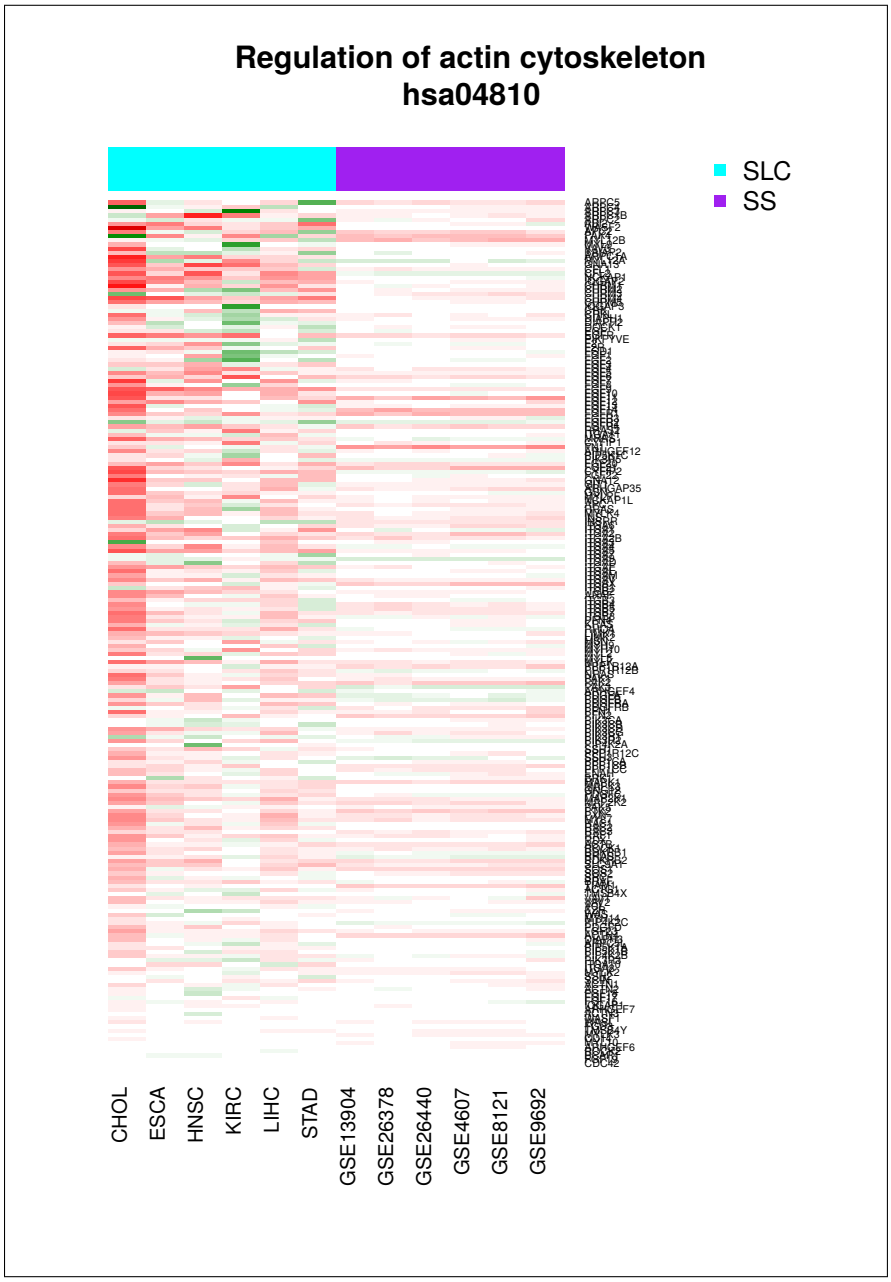
Supplementary Figure S7: [RIG-I- like pathway hsa04622] was observed to be uniformly up-regulated in both SS and SLC.



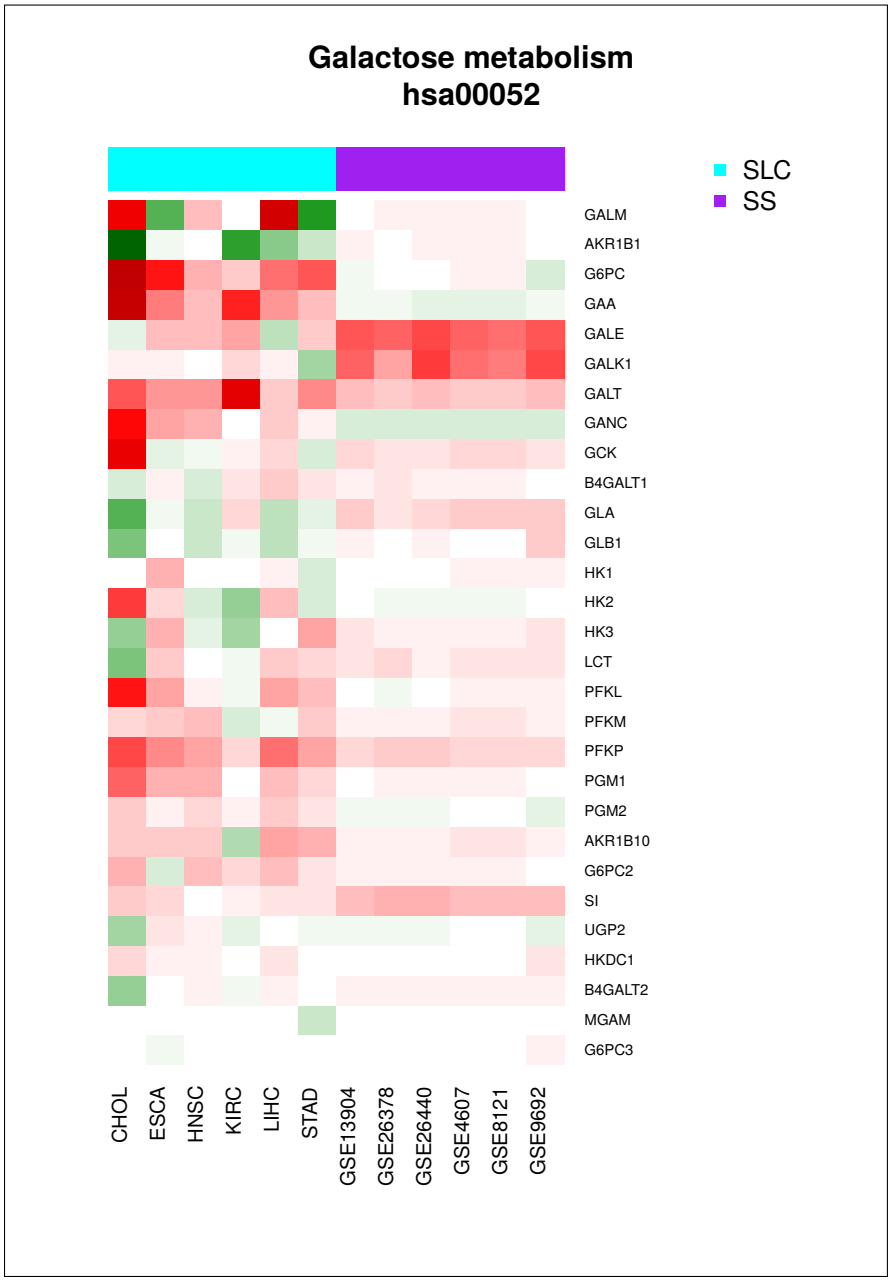
Supplementary Figure S8: [Bacterial invasion of epithelial cells hsa05100] Many pathogenic bacteria can invade phagocytic and non-phagocytic cells and colonize them intracellularly, then become disseminated to other cells. These bacteria use type III secretion systems to inject protein effectors that interact with the actin cytoskeleton.



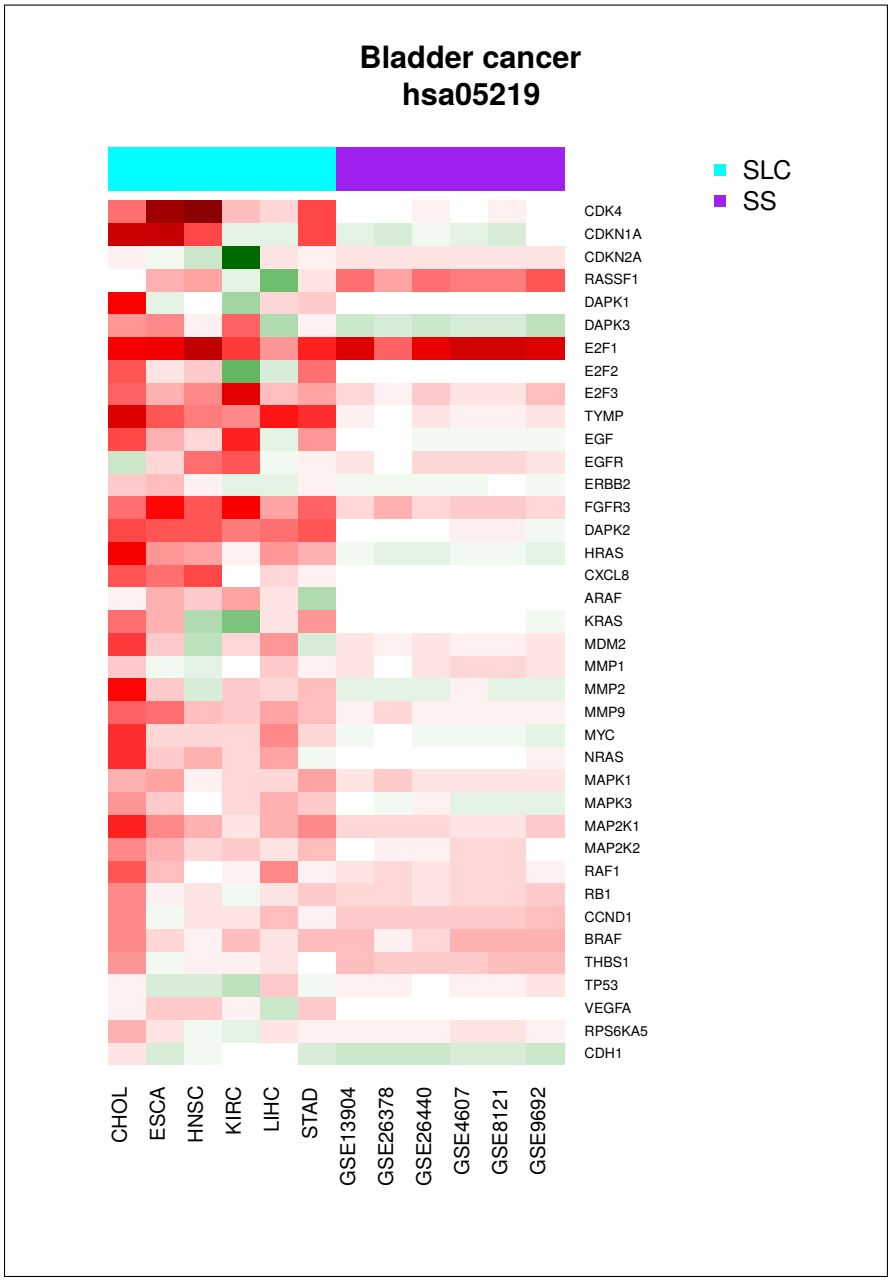
Supplementary Figure S9: [Epithelial cell signaling in *Helicobacter pylori* infection hsa05120] was observed to be up-regulated in both SS and SLC.



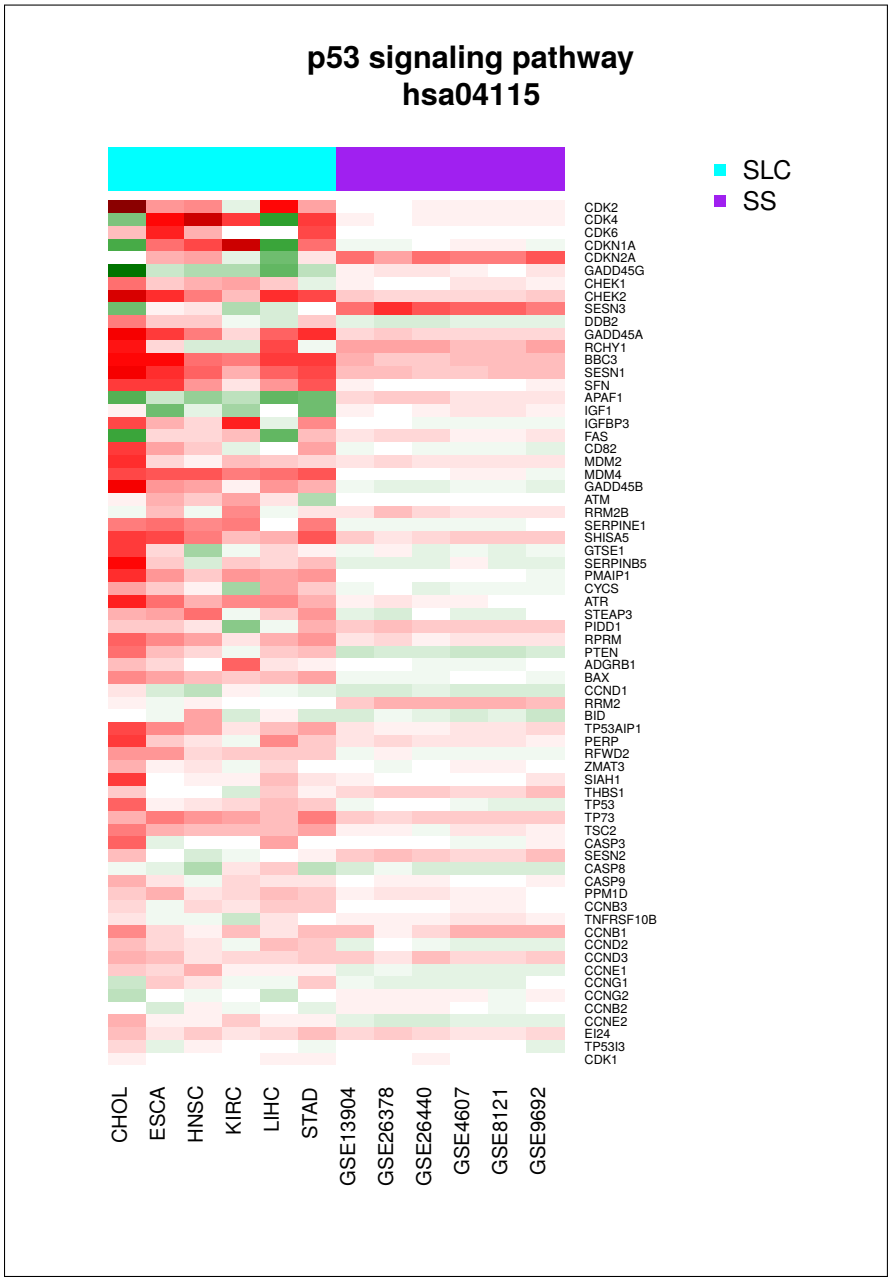
Supplementary Figure S10: [Regulation of Actin cytoskeleton pathway hsa04810] was observed to be altered in same direction in SLC as in SS. In leukocyte transendothelial migration the cells need to rearrange their cellular structure in order to squeeze through the transendothelial barrier. In cancer the cells need to rearrange themselves to relocate during metastasis.



Supplementary Figure S11: [Galactose metabolism pathway hsa00052] was observed to be up-regulated in both SS and SLC.



Supplementary Figure S12: [Bladder cancer pathway hsa05219] was observed to be up-regulated in both SS and SLC.



Supplementary Figure S13: [p53 signaling pathway hsa04115] was observed to be up-regulated in both SS and SLC.

Supplementary Figure S14: [Ribosome biogenesis in eukaryotes pathway hsa03008] was observed to be in different direction in SS and SLC. This pathway was found uniformly up-regulated in SLCs but is uniformly down-regulated in SS.