Integrated analysis of gene correlation reveals disordered relationship between metabolism and immunity in tumor microenvironment

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1 **Abstract:**

2 **Background:** Metabolism reprogramming and immune evasion are the
3 most fundamental hallmarks for cancer survival. The complex
4 interactions between metabolism and immune systems in tumors and
5 their microenvironment is complicated. Researching on the correlation
6 changes between metabolic and immune related-genes in normal and
7 tumor tissues would help to reveal these complex interactions.

8 **Methods:** In this study, the mRNA profiles across 11 cancer types was
9 obtained from The Cancer Genome Atlas (TCGA). Then, the spearman's
10 correlation coefficient was calculated between metabolic and immune
11 related-genes for each sample group.

12 **Results:** Our results showed that the number of correlated gene pairs was
13 reduced significantly in tumor tissues compared with those of normal
14 tissue, especially in KIRC, KIRP and STAD. Functional enrichment
15 analysis for the universal (the pairs appeared in more than 2 cancer types)
16 and specific (the pairs only in one specific cancer type) gene pairs across
17 cancer types revealed top pathways which appeared in tumor and normal
18 samples, such as phosphatidylinositol signaling system and inositol
19 phosphate metabolism. Thereinto, the pairs in normal tissues missing in
20 tumors may indicate they are important factors affecting immune system,
21 such as, DGKs and PIP4ks. The correlation analysis between immune
22 checkpoint and metabolism genes also showed a reduced correlation in

23 tumor and had the tissue specificity, such as, *FUT8* was strongly
 24 correlated with *PDCDI* in the HC of STAD and they had a weaker
 25 correlation in other normal tissues and tumor types.

26 **Conclusions:** Our study provides a novel strategy for investigating
 27 interaction of tumor immune and metabolism in microenvironment and
 28 offers some key points for exploring new targets including metabolic
 29 targets and immunomodulator of immune checkpoints .

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31 **Keywords:** spearman correlation, metabolism, immunity, pan-cancer,
 32 immune checkpoints

33 **Background**

34 Recently, inflammation and immune evasion are considered as hallmarks
 35 of cancer progression, highlighting the direct involvement of immune
 36 cells^[1, 2]. The study on immunology has made great progress in cancer
 37 treatment, and the role of immune cells in cancer progression is
 38 well-recognized^[3, 4]. Cancer immunotherapy has subverted the traditional
 39 concept of treatment, such as immune checkpoint inhibitors, cancer
 40 vaccines and chimeric antigen receptor redirected T (CAR-T) cell
 41 therapy^[5-8].

42 The research progresses on cancer has indicated that metabolic
 43 reprogramming is another hallmark of cancer^[9]. Cells that are common to
 44 many cancers that do not produce enough energy due to lack of oxygen,
 45 carbohydrate or protein use altered metabolic pathways to ensure their
 46 survival. Thus, malignant cells acquire the molecular materials and
 47 energy necessary to sustain proliferation through unusual metabolic
 48 pathways. The tendency of malignant cells to utilize glucose via the
 49 process of glycolysis irrespective of the oxygen availability is known as
 50 "aerobic glycolysis" which was pointed by Otto Warburg^[10, 11].

51 Though metabolic reprogramming and evasion of immune surveillance
 52 are distinct processes, recent evidence has accumulated to show that
 53 specific metabolic signatures as an important regulator in both innate and
 54 adaptive immunity in human cancer^[12]. The immune response is

55 associated with dramatic modifications in tumor microenvironment (TME)
56 metabolism, including depletion of nutrients, increased oxygen
57 consumption, and the generation of reactive nitrogen and oxygen
58 intermediates^[13]. For example, the bile metabolism of bile acids in gut
59 microbiome can influence the NKT cell-driven killing ability^[14].
60 Similarly, depletion in amino acid and nutrient depletion were reported
61 for T cells functions on anti-tumor effect^[15]. Besides, the crosstalk
62 between immune cells and other cells could influence the immunity^[16, 17].
63 Taken together, the findings of immune cells function have been showed
64 altered by the TME extensively in cancers.

65 The metabolism and immunity are fundamentally linked among the
66 malignant cell, immune cell and the microenvironment cells around them.
67 Thus, there is a renewed interest to exploit the link between these two
68 cancer-related processes in order to develop potent anticancer
69 therapeutics. The correlation between genes expression level can reflect
70 the relation between biological processes^[18-20]. Up to now, some
71 researchers have excavated and studied metabolic^[21, 22] and immune^[23, 24]
72 related genes separately based on TCGA database, however, few studies
73 has been done on the expression relationship between metabolic and
74 immune related genes in tumor samples. Thus, there is an interest to
75 exploit the link between these two cancer-related processes in order to
76 develop potent anticancer therapeutics. In this study, we analyzed 11

different cancers from the TCGA database, including 5645 cancer and normal samples, describing the co-expression relationship between metabolic and immune genes in pan-cancer. Our findings will provide a comprehensive data basis for further studies of metabolic and immune associations in tumor tissue.

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89 **Methods**

90 **Data obtaining and preprocessing**

The mRNA expression data and clinical information data were obtained from The Cancer Genome Atlas (TCGA) program through the NCI's Genomic Data Commons (GDC) website (<https://gdc.cancer.gov/>)^[25]. 5645 samples across 11 cancers were included in this research. Samples of colon adenocarcinoma (COAD) and rectum adenocarcinoma (READ) were mixed into colorectal carcinoma (CRC), thus left 10 cancer types in the following study. According to the clinical information, each cancer

was first classified into 5 groups: healthy control (HC) and tumor samples from stage 1 to stage 4. Then stage 1 and stage 2 were merged into early stage, while stage 3 and stage 4 were merged into advanced stage, the sample details was presented in Supplement Table 1.

The list of all human cellular metabolism genes was downloaded from ccmGDB^[26]. The immune-related gene list was obtained from the Reactome database, these genes were grouped according to three pathways in Reactome Pathway Browser(<http://www.reactome.org/>): adaptive immunity, innate immunity and cytokine signaling pathway in immune system^[27, 28]. The genes were listed in Supplement Table 2.

For the RNA-seq data, raw read counts were used to calculate TPM^[29-31]. Annotation file from GENCODE (<https://www.gencodegenes.org/>)^[32] was used to calculate the length of each gene, which was needed in the calculation of TPM. For each gene, mean TPM was calculated, gene with mean TPM less than 1 was considered as low expressed gene and filtered out.

Co-expression analysis and DEG analysis

Python (<https://www.python.org/>) and the “spearmanr” function in package scipy (<https://www.scipy.org/>) were used to calculate Spearman’s rank correlation in our study^[33].

118 For each cancer type, co-expression analysis was conducted in 7 groups
 119 of samples: normal control, tumor from stage 1 to stage 4, early stage
 120 tumor, advanced stage tumor. In each sample group, all metabolism genes
 121 were paired with 3 groups of immune-related genes: adaptive immunity,
 122 innate immunity and cytokine signaling pathway, respectively. There
 123 were 21 co-expression analyses to be conducted for each cancer. To
 124 calculate Spearman's r value, TPM values of these pairs in each sample
 125 were used. Pairs with $|r| > 0.8$ were determined as strongly co-expressed
 126 pairs^[34]. In order to present the landscape when applying different
 127 threshold values to our data, the number of co-expressed pairs with $|r| >$
 128 0.7 , $|r| > 0.8$ and $|r| > 0.9$ was counted.

129 In order to clarify the differences between normal and tumor tissues, a
 130 further filter was conducted between HC with tumor (early and advanced
 131 stage). Pairs with $|r_{\text{hc}}| > 0.8$ in HC and $|r_{\text{tumor}}| < 0.4$ were defined as HC
 132 specific metabolism-immune gene pairs. Pairs with $|r_{\text{hc}}| < 0.4$ in HC and
 133 $|r_{\text{tumor}}| > 0.8$ in tumor (in early or advanced stage or in both) were
 134 defined as tumor specific metabolism-immune gene pairs. For these pairs,
 135 the number of cancers which they existed in was counted to define the
 136 universality and specificity pairs. The pairs involved in only one cancer
 137 were defined as specificity. Otherwise, they were universality pairs.
 138 Differentially expressed gene(DEG) analysis was performed using
 139 DEseq2^[35], raw counts of genes was used and pvalue was corrected by

140 IHW method^[36]. Genes with fold change greater than 1.5 and adjusted p
141 value less than 0.05 was considered as DEGs.

142 **Functional analysis**

143 An R package “clusterProfiler” was used to perform KEGG (Kyoto
144 Encyclopedia of Genes and Genomes) and GO (Gene Ontology) analysis
145 on the universality and specificity genes pairs^[37]. The R package “heatmap”
146 was used to draw the heatmap.

147 **Results**

148 **Statistics of data**

149 Totally, 5645 samples in 10 cancer groups were included in our research.
150 Each group was divided into 7 groups: healthy control, tumor with
151 clinical stage 1 to stage 4, early stage tumor (tumor clinical stage 1 and
152 stage 2), advanced stage tumor (tumor clinical stage 3 and stage 4). For
153 each group, correlation coefficient values were calculated between
154 immune and metabolism related genes. Co-expressed pairs were further
155 filtered into 3 groups: 0.7 ($|r| > 0.7$), 0.8 ($|r| > 0.8$) and 0.9 ($|r| > 0.9$). The
156 number of pairs in these groups were counted and presented in Figure 1,
157 Figure 2 and Supplement Table 3. The number of co-expressed
158 metabolism-immune pairs in HC is several to hundreds of times as many
159 as that in tumors, except THCA. In THCA case, HC and tumor had the

160 same order of magnitude of pairs number in $|r| > 0.8$ and $|r| > 0.7$ group,
 161 the number that in some tumor stages was even slightly more than that in
 162 HC. In addition, the co-expressed gene pairs show differences among
 163 cancers. In KIRC, KIRP and STAD, the co-expressed gene pair numbers
 164 was dozens of times greater than other cancers in quantity. Besides, the
 165 particular gene pairs were great different between HC and tumors.

166 **Universality and specificity of cancer types**

167 Further filter was conducted to find out universal and specific
 168 metabolism-immune gene pairs across various types of cancer. The
 169 number of HC specific pairs was also much more than tumor specific
 170 pairs. The gene pairs were then combined with the number of their
 171 occurrences in cancer types. The pairs involved in only one cancer were
 172 defined as specific, otherwise, they were universal pairs. The full result is
 173 presented in Supplement Table 4-7. The top universal
 174 metabolism-immune gene pairs in HC and tumor were presented in Table
 175 1 and Table 2, respectively. In HC group, several universal co-expressed
 176 gene pairs existed in more than 4 tissues, for example: *MED4-ACTR10*,
 177 *LSM5-ACTR10* and *NUPL2-UBA5*, etc. In the mean time, top universal
 178 co-expressed genes existed in more than 3 tumor tissues, these pairs are:
 179 *ADH1B-C7*, *GNG2-PTPRC*, *GNGT2-LAIR1*, *GNGT2-IL16*,
 180 *INHBA-COL3A1* and *CD38-PTPN7*.

181 **Functional analysis**

182 In order to find out biological meanings of universal and tissue-specific
 183 metabolism-immune gene pairs, KEGG and GO enrichment analysis was
 184 performed in two system genes, respectively. Specific pairs of each
 185 cancer and universal pairs were separated to perform analysis, the
 186 grouping was similar with before: gene pairs were grouped according to 3
 187 groups of immune genes, for each group, metabolism genes and immune
 188 genes were separated to perform KEGG and GO analysis. KEGG and GO
 189 terms derived from specific pairs were presented in Supplement Table 8-11.
 190 The function annotation of universal pairs were presented in Supplement
 191 Table 12-15.

192 In KEGG and GO analysis, there were pathways which had a higher
 193 frequency of occurrence in cancer types, which means although different
 194 cancer types had differently co-expressed metabolism-immune gene pairs,
 195 these genes tended to show up in several same pathways. The pathways
 196 enriched to the most cancer types were screened out as the top KEGG and
 197 GO terms and shown in Supplement Table 16. There were also existed
 198 specific pathways indicating the pairs enriched in different pathways
 199 depending on the tumor types. Several metabolism pathways only showed
 200 in STAD and LUSC, respectively.

201 In the specific gene pairs group, the top KEGG pathways were

phosphatidylinositol signaling system and inositol phosphate metabolism which appeared in 6 tumor types and in 10 normal tissues. Notably, the pathway including DGKs and PIP4Ks genes was only showed in normal types. In the universality gene pairs group, most genes strongly correlated with immune related genes were enriched in purine metabolism in normal tissues. Correspondingly, apelin signaling pathway including most metabolism genes was showed in tumor samples. The immune related genes were also analyzed by the functional enrichment and showed in Supplement Table 12-15.

The correlation between immune checkpoint and metabolism genes

To better understand the potential role of dysregulated gene pairs in immunotherapy, we choose the targetable immune checkpoint genes *TNFRSF4*, *CTLA4*, *PDCD1*, *CD274* and analysed the correlation between them and metabolism genes. We observed the tissue specificity and a consistent change that the correlation coefficient was much higher in normal than in tumor. Strong correlation between metabolism genes and immune checkpoint genes was showed in the one normal tissue or some HC of STAD, KIRP, KIRC and BRCA. Except for these four tissues, the correlation is not strong across other normal types($r > 0.8$ as strong correlation). Looking at individual immune checkpoint genes, the most metabolism genes were correlated with *CD274* in the normal tissues of

LIHC, KIRP, KIRC and BRCA. Only three genes were strongly correlated with *CTLA4* in HC of THCA and STAD. The genes correlated with *TNFRSF4* mainly enriched in normal tissues of STAD, the individuals were in HC of THCA(Figure 3A).

In order to rationalize the importance of above immune checkpoint related-genes in tumor, the differential expression levels were analysed. *CD274* was up-expressed in STAD, HNSC, KIRP and KIRC. *CTLA4* was up-regulated across cancer types except THCA. *PDCDI* was up-regulated in 5 cancer types. In the metabolism genes, *ADA*, *CA9*, *CHPF*, *IL4I1* and *PYCR1* were all up-regulated at least 7 cancer types. The changed correlation may not mainly due to altered gene expression between normal and tumor, such as, *TNFRSF4* and *MUC6* was strongly correlated in normal tissues of STAD, differential expression of *TNFRSF4* or *MUC6* was not observed in STAD(Figure 3B).

Discussion

Cancers are not just masses of malignant cells but including extracellular components and stromal cells, the malignant and these non-malignant cells and their cell-to-cell signalling creat the TME. However, the crosstalks between them is complicated and affected by a lot of factors. Our study was focused to elucidate the intertwined links between cell metabolism and tumor immunity through related-genes' correlation

244 analysis in human cancer. The results indicated that the disordered
245 interaction occurred between metabolism and immunity in the TME at the
246 overall level. Our study also revealed most relevant pairs and most
247 significant signal pathway turbulences at the gene level in normal and
248 cancer types. Besides, the metabolism genes strongly correlated with
249 immune checkpoint genes were enumerated which may provide the
250 potential therapeutic targets.

251 Many important stroma cells interact each other dynamically, with
252 expressing metabolic and some immune genes, which have been reported
253 to have critical roles in the immune response in cancer tissues^[38]. The
254 correlation among genes in tissue might reflect important biological
255 relationship and variation in TME. The number of strongly correlated
256 metabolism-immune gene pairs in normal tissues is 2-200 times than in
257 tumor and even in tumor stage 1, revealing the disordered relation
258 occurred in early stages of tumor and during the development of cancer.
259 The TME turbulence may be the main reason leading the correlation
260 changes of gene pairs, such as, cancer cells are frequently surrounded by
261 hypoxic and acid microenvironment, to survive, they have evolved
262 multiple adaptations and summoned other cells to help them^[39, 40].
263 Besides much alteration has emerged in immune cells and immune
264 responses as well^[41]. Thus, it's easy to understand the changes of their
265 correlation between tumor and normal samples. The signatures of

immune and metabolism vary depending of different cancer and tissues^[42], including macrophages quantity, overall lymphocyte infiltration, glycolysis and other biological characteristics. In particular types, we found that the number of gene pairs was varied across normal and tumor tissues. This may be a consequence of different environments in the different tissues.

The specific metabolic genes which strongly correlated with immune genes are enriched in some same pathways across both in normal and cancer tissues. The phosphatidylinositol signaling system and inositol phosphate metabolism have been identified in all normal tissues and 6 cancer types. The alteration of above pathways including gene expression, mutation, and posttranslational modification on key phospholipases and kinases are not only observed in tumor cells^[43, 44] but also in immune cells^[45, 46] and stroma cells^[47, 48], control the switch between immune stimulation and suppression in TME, which indicates phosphatidylinositol signaling system and inositol phosphate metabolism are closely relevant with immunoregulation in cancer. The metabolic genes in the two pathways which strongly correlated with immune genes in normal but missing in tumor may be the key gene connecting to immune disorder and tumor escape in tumor, such as, DGKs and PI5P4Ks family. DGKs family are considered as physiologic regulators of T- and NK-cell development and function through regulating TCR-signaling.

288 CD8-T cells and NK-cell activity can be enhanced and immune escape
 289 can be prevented through inhibition of DGKA in CRC^[49]. *DGKA* was
 290 up-regulated in KIRP, KIRC and LUAD, in our research, *DGKA* may
 291 represent an key immunomodulator influencing the activity of immunity
 292 of immune cells in these TME. mTORC1 signaling influences the
 293 immune system by regulating diverse immune cell types, The enzyme
 294 encoded by *PIP4K2C* gene is a substrate of mTORC1^[50] and negatively
 295 regulates mTORC1, which indicate a close relationship between
 296 *PIP4K2C* and immune cells. Pip4k2c knockdown mice displayed
 297 increased immune infiltrates in various tissues, including liver, intestine,
 298 kidney, and lungs, these infiltrating cells are mostly T cells and B cells^[51].
 299 *PIP4K2C* was up-regulated in BRCA, which suggest *PIP4K2C* may
 300 influence the immune infiltrates in BRCA. Thus, the correlation analysis
 301 between metabolic genes and immune genes could identify some key
 302 metabolic genes which have impact on immune systems, however, how
 303 the other key metabolic genes (Supplement Table 17) influence the
 304 immune systems, we need further studies.

305 We observed the correlation was much higher in normal than in tumor
 306 between metabolism genes and immune checkpoint genes. The strongly
 307 related pairs were mainly showed in the HC of STAD, KIRP, KIRC and
 308 BRCA, and the specific correlation varied across normal tissues. These
 309 results suggest that correlation between immune checkpoints and

metabolism genes is tissue specific and have disordered in tumors. We find a positive correlation between *HIF1A* and *PDL1* expression across cancers but not as strong as in normal tissues. *HIF1A* can regulate *PDL1* expression at both the mRNA and protein level in myeloid-derived suppressor cells and also confirmed in melanoma and breast carcinoma bearing mice^[52]. *HIF1A* was up-regulated in LUAD and LUSC from our data suggesting it may participate in *PDL1* regulation in these two cancers. Study shows that *FUT8* is the a post-translational regulator of *PDCD1* expression and blocking *FUT8* can enhance T cell activation under antigen presentation^[53]. *FUT8* had a close relationship with *PDCD1* in HC of STAD while the correlation was decreased in tumors and it had a higher expression in LUAD, LUSC, CRC and BRCA, which indicates *FUT8* may influence immunity of T cell by affecting *PDCD1* expression in these tumor types. *IL4I1* is the most described member of a family of immunosuppressive enzymes produced by antigen presenting cells which may act on T cells by direct inhibition of effector cell proliferation^[54]. In this study, *IL4I1* is correlated with *TNFRSF4* in most cancer and normal types and up-regulated in all cancer types except STAD. Highly expressed *IL4I1* may play an important role in immune escape by affecting immune cell through *TNFRSF4*. Future research is needed to focus on the verification of these gene pairs associated with immune checkpoints.

332 **Conclusion**

333 The disturbance of correlation between metabolism and immune related
 334 genes is revealed in our study. Phosphatidylinositol signaling system and
 335 inositol phosphate metabolism are strongly correlated with immune
 336 system both in normal and cancer tissues. The metabolic genes in the two
 337 pathways which strongly correlated with immune genes in normal but
 338 missing in tumor, such as DGKs and PI5P4Ks family, may be the key
 339 gene connecting to immune disorder and tumor escape in tumor.
 340 Particularly, the metabolic genes, such as, *HIF1A* and *FUT8* correlated
 341 with immune checkpoint are screened out which may be potential
 342 immunomodulator influencing therapeutic effect. The results including
 343 strongly correlated pairs and their pathways may open new avenues for
 344 exploration of the mechanisms underlying metabolism reprogramming
 345 and immune escape in TME.

346 **List of abbreviations**

347 HNSC: Head and Neck Squamous Cell Carcinoma

348 KIRC: Kidney Renal Clear Cell Carcinoma

349 KIRP: Kidney Renal Papillary Cell Carcinoma

350 LIHC: Liver Hepatocellular Carcinoma

351 LUAD: Lung Adenocarcinoma

- 352 LUSC: Lung Squamous Cell Carcinoma
- 353 STAD: Stomach Adenocarcinoma
- 354 THCA: Thyroid Carcinoma.
- 355 BRCA: Breast Invasive Carcinoma
- 356 CRC: Colorectal Carcinoma
- 357 HC: Healthy Control
- 358 TCGA: The Cancer Genome Atlas
- 359 TME: Tumor Microenvironment
- 360 DGKs: Diacylglycerol Kinases
- 361 PIP4ks: Phosphatidylinositol-5-Phosphate 4-Kinases
- 362 FUT8: Fucosyltransferase 8
- 363 TNFRSF4: TNF Receptor Superfamily Member 4
- 364 CTLA4: Cytotoxic T-Lymphocyte Associated Protein 4
- 365 PDCD1: Programmed Cell Death 1
- 366 CD274: Programmed Cell Death Ligand 1
- 367 DGKA: Diacylglycerol Kinase Alpha
- 368 PIP4K2C: Phosphatidylinositol-5-Phosphate 4-Kinase Type 2 Gamma

369 HIF1A: Hypoxia Inducible Factor 1 Subunit Alpha

370 IL4I1: Interleukin 4 Induced 1

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373 **Declarations**

374 **Ethics approval and consent to participate**

375 No ethical approval was required for this study.

376 **Consent for publication**

377 Not applicable.

378 **Availability of data and material**

379 Data of all considered in TCGA are publicly available at The Genomic

380 Data Commons Data Portal (<https://portal.gdc.cancer.gov/>).

381 The datasets supporting the conclusions of this article are included within

382 the article (and its additional files).

383 **Competing interests**

384 The authors declare that they have no competing interests.

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390 **Authors' contributions**

391 Hongli Du designed and conceived study. Zixi Chen conducted analyses.

392 Jinfen Wei and Zixi Chen wrote the manuscript. Yuchen Yuan and Ying

393 Cui assisted with conducting analyses the data. Yanyu Zhang and Yumin

394 Hu offered suggestions for the analysis and manuscript writing. Hongli

395 Du were involved in revising the manuscript. All authors read and

396 approved the final manuscript.

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540 **Figure legend**

541 Figure 1. Flow chart of the integrated analysis of co-expressed pairs

542 The mRNA expression data were obtained from The Cancer Genome

543 Atlas (TCGA). 5645 samples across 11 cancers were included in this

544 research. According to the clinical information, each cancer was

545 classified into 5 groups: healthy control (HC) and tumor samples from

546 stage 1 to stage 4. For each gene with mean TPM less than 1 was filtered

547 out. The list of human cellular metabolism genes was downloaded from

548 ccmGDB. The immune-related gene list was obtained from the Reactome

549 database. The spearman's rank correlation was calculated between

550 immune and metabolism genes and the differential gene analysis was

551 analysed between HC and tumor samples. The correlation pairs with

552 $|r_{hc}| > 0.8$ in HC and $|r_{tumor}| < 0.4$ were defined as HC specific

553 metabolism-immune gene pairs. Pairs with $|r_{hc}| < 0.4$ in HC and

554 $|r_{\text{tumor}}| > 0.8$ in tumor were defined as tumor specific pairs. The pairs
 555 including immune checkpoint were the immune checkpoint pairs. Besides,
 556 the pairs involved in only one cancer were defined as specific, otherwise,
 557 they were universal pairs. Then KEGG and GO enrichment analysis was
 558 performed between these pairs and the key pairs were screened out.

559

560 Figure 2. The quantitative comparison of co-expressed gene pairs between
 561 HC and tumor early/advanced stage tissues.

562 Fig.abc, Fig.def and Fig.ghi represent comparison among HC, early and
 563 advanced stage in result of level0.7, level0.8 and level0.9 respectively.

564 Fig.adg, Fig.beh and Fig.cfi represent comparison among HC, early and
 565 advanced stage in result of adaptive immunity, cytokine signaling
 566 pathway and innate immunity groups, respectively. The data of three
 567 levels all reflect the same rule that co-expressed gene pairs in HC are
 568 much more than those in early and advanced stages among all types of
 569 cancer, the proportions of HC, early and advanced stage are different
 570 among cancers.

571

572 Figure 3. The quantitative comparison of co-expressed gene pairs
 573 between HC and tumor s1 to s4 stage tissues.

574 The literal explanations also see in Figure 2.

575

Figure 4. Hierarchical clustering of immune checkpoint gene pairs.

Fig.a is the spearman correlation heatmap of metabolic genes with immune checkpoint genes. Red color illustrates a very strong positive correlation ($r > 0.8$), white represents missing value, and blue represents a negative correlation ($r < -0.8$).

Fig.b represents the clustering of genes in the immune checkpoint gene pairs. Red color illustrates the gene was up-regulated in tumor compared with normal tissues(fold change > 1.5), white represents non-significant difference, blue represents gene was down-regulated (fold change < -1.5).

Table 1. Top universal metabolism-immune gene pairs in HC and tumors

	Metabolism	Immune	Normal tissue	Count	Group
Normal tissues	<i>MED4</i>	<i>ACTR10</i>	BRCA,LUSC,CRC,STAD	4	adaptiveimmune
	<i>LSM5</i>	<i>ACTR10</i>	BRCA,LUSC,KIRP,KIRC	4	adaptiveimmune
	<i>NUPL2</i>	<i>UBA5</i>	BRCA,LUSC,KIRC,STAD	4	adaptiveimmune
	<i>PLCB1</i>	<i>FBXL7</i>	LUAD,LUSC,CRC,STAD	4	adaptiveimmune
	<i>TEAD2</i>	<i>PJA1</i>	KIRP,CRC,KIRC,STAD	4	adaptiveimmune
	<i>PSMB1</i>	<i>CAPZA2</i>	LUSC,KIRP,KIRC,STAD	4	adaptiveimmune
	<i>POLR2K</i>	<i>DCTN6</i>	HNSC,LUSC,CRC,STAD	4	adaptiveimmune
	<i>GNAS</i>	<i>PDAP1</i>	KIRC,STAD,CRC,KIRP	4	innate immune
	<i>MED4</i>	<i>ACTR10</i>	STAD,BRCA,CRC,LUSC	4	innate immune
	<i>EXOSC8</i>	<i>ATG5</i>	KIRC,BRCA,LUSC,KIRP	4	innate immune
	<i>PSMB1</i>	<i>CAPZA2</i>	KIRC,STAD,LUSC,KIRP	4	innate immune
	<i>MOCS2</i>	<i>GOLGA7</i>	LUAD,STAD,CRC,LUSC	4	innate immune
	<i>LSM5</i>	<i>ACTR10</i>	KIRC,BRCA,LUSC,KIRP	4	innate immune

	<i>GNGT2</i>	<i>LAIR1</i>	<i>KIRP, BRCA, LUSC</i>	3	<i>adaptive immune</i>
	<i>INHBA</i>	<i>COL3A1</i>	<i>THCA, LUSC, LUAD</i>	3	<i>adaptive immune</i>
	<i>INHBA</i>	<i>COL1A2</i>	<i>THCA, LUSC, LUAD</i>	3	<i>adaptive immune</i>
	<i>INHBA</i>	<i>COL1A2</i>	<i>THCA, LUSC, LUAD</i>	3	<i>adaptive immune</i>
Tumor	<i>GNG2</i>	<i>PTPRC</i>	<i>CRC, STAD, LUAD</i>	3	<i>adaptive immune</i>
types	<i>PIK3R5</i>	<i>PRKCB</i>	<i>CRC, KIRC, LUSC</i>	3	<i>adaptive immune</i>
	<i>INHBA</i>	<i>COL1A2</i>	<i>LUAD, LUSC, THCA</i>	3	<i>cytokine signaling</i>
	<i>GNGT2</i>	<i>IL16</i>	<i>LUSC, KIRC, KIRP</i>	3	<i>cytokine signaling</i>
	<i>CD38</i>	<i>PTPN7</i>	<i>KIRC, BRCA, KIRP</i>	3	<i>cytokine signaling</i>
	<i>ADH1B</i>	<i>C7</i>	<i>BRCA, LUSC, KIRC</i>	3	<i>innate immune</i>
	<i>GNG2</i>	<i>PTPRC</i>	<i>STAD, CRC, LUAD</i>	3	<i>innate immune</i>
	<i>GNGT2</i>	<i>LAIR1</i>	<i>BRCA, KIRP, LUSC</i>	3	<i>innate immune</i>

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593 Additional files

594 Additional file 1 - Sample size of each group.xlsx

595 Additional file 2 - Gene list of four groups(metabolism, innate immunity, adaptive immunity,
596 cytokine signaling pathways).xlsx

597 Additional file 3 - Full table of statistics(The number of pairs with $|r| > 0.9, 0.8, 0.7$ in each group
598 across cancer types).xlsx

599 Additional file 4 - Universalilty pairs in HC group(the pairs in at least 2 normal tissues).xlsx

600 Additional file 5 - Specifity pairs in HC group(the pairs in only 1 normal tissue).xlsx

601 Additional file 6 - Universalilty pairs in tumor group(the pairs in at least 2 cancer types).xlsx

602 Additional file 7 - Specifity pairs in tumor group(the pairs in only 1 cancer type).xlsx

603 Additional file 8 - GO terms derived from specifity pairs in HC.xlsx

604 Additional file 9 - KEGG terms derived from specifity pairs in HC.xlsx

605 Additional file 10 - GO terms derived from specifity pairs in tumor.xlsx

606 Additional file 11 - KEGG terms derived from specifity pairs in tumor.xlsx

607 Additional file 12 - GO terms derived from universality pairs in HC.xlsx

608 Additional file 13 - KEGG terms derived from universality pairs in HC.xlsx

609 Additional file 14 - GO terms derived from universality pairs in tumor.xlsx

610 Additional file 15 - KEGG terms derived from universality pairs in tumor.xlsx

611 Additional file 16 - Top pathways counts(the pathways in most tissues and cancer types).xlsx

612 Additional file 17- The genes involved in phosphatidylinositol signaling system and inositol
613 phosphate metabolism across normal and cancer types.xlsx

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