



Alteration in Gene Pair Correlations in Tryptophan Metabolism as a Hallmark in Cancer Diagnosis

Meena Kishore Sakharkar¹ , Sarinder Kaur Dhillon²,
Karthic Rajamanickam¹, Benjamin Heng³ , Nady Braidy^{3,4} ,
Gilles J. Guillemin³  and Jian Yang¹

¹Drug Discovery and Development Research Group, College of Pharmacy and Nutrition, University of Saskatchewan, Saskatoon, SK, Canada. ²Institute of Biological Sciences, Faculty of Science, University of Malaya, Kuala Lumpur, Malaysia. ³Neuroinflammation Research Group, MND Research Centre, Department of Biological Sciences, Faculty of Medicine and Health Sciences, Macquarie University, Sydney, NSW, Australia. ⁴Centre for Healthy Brain Ageing, School of Psychiatry, University of New South Wales, Faculty of Medicine, Sydney, NSW, Australia.

International Journal of Tryptophan Research
Volume 13: 1–10
© The Author(s) 2020
Article reuse guidelines:
sagepub.com/journals-permissions
DOI: 10.1177/1178646920977013


ABSTRACT: Tryptophan metabolism plays essential roles in both immunomodulation and cancer development. Indoleamine 2,3-dioxygenase, a rate-limiting enzyme in the metabolic pathway, is overexpressed in different types of cancer. To get a better understanding of the involvement of tryptophan metabolism in cancer development, we evaluated the expression and pairwise correlation of 62 genes in the metabolic pathway across 12 types of cancer. Only gene *AOX1*, encoding aldehyde oxidase 1, was ubiquitously downregulated. Furthermore, we observed that the 62 genes were widely and strongly correlated in normal controls, however, the gene pair correlations were significantly lost in tumor patients for all 12 types of cancer. This implicated that gene pair correlation coefficients of the tryptophan metabolic pathway could be applied as a prognostic and/or diagnostic biomarker for cancer.

KEYWORDS: Tryptophan metabolism, cancers, TCGA, differentially expressed gene, gene pair correlation

RECEIVED: September 16, 2020. **ACCEPTED:** November 2, 2020.

TYPE: Original Research

FUNDING: The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: Dr Heng have been supported by a research grant (Scott Canner Young Researcher grant) from Tour de Cure Foundation and a research donation from the philanthropy group, Fight on the Beaches. Prof Guillemin is funded by the National Health and Medical Research Council (NHMRC) and Macquarie University.

DECLARATION OF CONFLICTING INTERESTS: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

CORRESPONDING AUTHORS: Meena Kishore Sakharkar, Drug Discovery and Development Research Group, College of Pharmacy and Nutrition, University of Saskatchewan, 107 Wiggins Road, Saskatoon, SK S7N 5E5, Canada. Email: meena.sakharkar@usask.ca

Jian Yang, Drug Discovery and Development Research Group, College of Pharmacy and Nutrition, University of Saskatchewan, 107 Wiggins Road, Saskatoon, SK S7N 5E5, Canada. Email: jian.yang@usask.ca

Introduction

Cancer is the second most-frequent cause of death worldwide, killing more than 8 million people each year.¹ According to Cancer Statistics 2020, it was estimated that there would be approximately 1 806 590 new cases in 2020.² Despite recent advances in diagnosis and therapies, prognosis remains poor for advanced-stage and recurrent cancer patients, partially due to cancer heterogeneity and resistance to therapies. Therefore, it is imperative to identify common and unique pathophysiological changes for different types of cancer, which, in turn, would facilitate the development of novel techniques for early diagnosis and more-efficient therapeutic regimens.

Compared to normal cells, cancer cells have undergone a significant alteration in metabolic functions to ensure their proliferation and sustenance.^{3,4} Reprogramming of cellular metabolism has been observed in cancer cells to maintain viability and building biomass.⁵ Furthermore, cancer cells can express abnormal antigens to evade host immunosurveillance.⁶ Being the building blocks of proteins, amino acids are of a high demand in fast proliferating cancer cells. Therapies targeting amino acid metabolism have been investigated.⁷ Tryptophan is an essential amino acid, and its

catabolism is involved in both immunomodulation and cancer development.^{8,9}

Indoleamine 2,3-dioxygenase (IDO1), which is a rate-limiting enzyme in tryptophan catabolism and breaks down tryptophan into kynurenine, is overexpressed in several types of cancer.^{10,11} IDO1 overexpression causes a decrease of tryptophan and an accumulation of kynurenine in local tumour environment, leading to suppressed immune function T-cell^{12–14} and natural killer cells.^{15,16} Activation of IDO1 also leads to the production of T regulatory cells¹⁷ and myeloid derived suppressor cells.¹⁸ The presence of these cells suppresses local immune surveillance to allow cancer cells to grow undetected. Serum kynurenine level has been proposed as a biomarker of cancer risk, cancer progression and poor prognosis in several malignancies.^{19–21} Furthermore, survival time was observed to be shorter for cancer patients with a low serum level of tryptophan along with a high level of neopterin.^{17,22,23} IDO1 has emerged to be one of the targets to develop novel anticancer agents. The interest in IDO1 inhibition has led to 34 clinical trials examining the use of IDO1 inhibitor as a first- or second-line immunotherapy treatment in metastasis cancer patients. Efficacy of IDO1 inhibitor monotherapies have been disappointing while the combination of IDO1 inhibitors with



Table 1. Number of patients in normal control, tumor (all stages combined) and each individual stage in 12 types of cancer.

| TYPE | NORMAL | TUMOR | I | II | III | IV | CANCER DESCRIPTION |
|-------|------------|-------------|-----|-----|-----|-----|---------------------------------------|
| BLCA | 19 | 414 | 2 | 129 | 138 | 130 | Bladder urothelial carcinoma |
| BRCA | 113 | 1102 | 170 | 585 | 234 | 18 | Breast invasive carcinoma |
| COAD | 41 | 471 | 72 | 166 | 119 | 57 | Colon adenocarcinoma |
| ESCA | 11 | 159 | 14 | 68 | 47 | 8 | Esophageal carcinoma |
| HNSC | 13 | 127 | 12 | 19 | 26 | 54 | Head and neck squamous cell carcinoma |
| KIRC | 72 | 538 | 249 | 50 | 116 | 69 | Kidney renal clear cell carcinoma |
| LIHC | 50 | 371 | 158 | 81 | 77 | 4 | Liver hepatocellular carcinoma |
| LUAD | 59 | 533 | 259 | 114 | 80 | 26 | Lung adenocarcinoma |
| PRAD | 52 | 498 | X | X | X | X | Prostate adenocarcinoma |
| STAD | 32 | 375 | 52 | 102 | 149 | 36 | Stomach adenocarcinoma |
| THCA | 58 | 502 | 254 | 47 | 103 | 52 | Thyroid carcinoma |
| UCEC | 23 | 551 | 332 | 50 | 121 | 27 | Uterine corpus endometrial carcinoma |
| TOTAL | 543 | 5641 | | | | | |

conventional anti-cancer treatments had mixed results. Three combination therapy trials (NCT02752074, NCT03386838 and NCT3417037) have shown to not improve progression-free survival in overall population as compared to mono-immune check point therapy while the remaining trials have shown promising synergistic antitumour therapeutic effects when used with other immunotherapeutic drugs in advanced cancer patients.

Carcinogenesis is a complicated and complex process and requires coordination of multiple genes. To get a better understanding of tryptophan metabolism in carcinogenesis and cancer progression, it is necessary to analyze all rather than an individual member such as IDO1. Recently, pairwise gene expression state was reported to be associated with cancer patient survival.²⁴ Our studies also showed that gene expression correlation coefficients could be used as a prognosis biomarker for human breast cancer.²⁵ Thus, in the current study, we evaluated the expression and gene pair correlation for 62 genes involved in tryptophan metabolism across 12 different types of cancer.

Materials and Methods

Data acquisition

A list of 62 genes involved in tryptophan metabolism was downloaded from PathCards database (<https://pathcards.genecards.org/>), which is an integrated database of human biological pathways and their annotations. RNAseq data for 12 types of cancer were downloaded from The Cancer Genome Atlas (TCGA) via the Genomic Data Commons (GDC) data portal. The numbers of control case, tumor

patient, and patient in each stage were summarized in Table 1. In total, 543 control cases and 5641 cancer patients were involved in the study. Sample sheet files were also downloaded to extract the clinical information. For each subject, the expression of 60483 RNA transcripts was analyzed in term of FPKM value.

Identification and visualization of differentially expressed genes

Differentially expressed genes (DEGs) in cancer (total and individual stages) against normal were identified using the DEGseq package from R.²⁶ Likelihood Ratio Test (LRT) was applied, and the sample expression profiles were screened using P -value $< .001$. The output was expressed in normalized \log_2 fold-change (Log_2FC). Expression changes of the 62 genes involved in tryptophan metabolism were then extracted for the 12 types of cancer (Table 2).

Computation of correlation matrix

Correlation matrix was computed for each type of cancer using the `cor` function in R. Visualization of the matrix was done using the `corrplot` function in R. Positive and negative correlations are represented in blue and red, respectively.

Protein-protein interaction (PPI) network

Human protein interactome (BIOGRID-ORGANISM-Homo_sapiens-4.0.189.tab) was downloaded from BioGRID database (<http://www.thebiogrid.org>).²⁷ PPI data was extracted for the 12 pairs of genes that were positively correlated in all 12

Table 2. Differential expression (log₂FC) of 62 genes in tryptophan metabolism across 12 different types of cancer.

| GENE NAME | BLCA | BRCA | COAD | ESCA | HNSC | KIRC | LHC | LUAD | PRAD | STAD | THCA | UCEC | FINAL |
|-----------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| AADAT | 0.74 | 0.05 | 1.42 | 0.99 | 0.38 | -0.44 | -2.09 | 0.00 | 0.86 | 0.58 | -0.28 | 0.42 | False |
| AANAT | 0.62 | 0.87 | 1.81 | 1.58 | 1.41 | 2.74 | 2.44 | 1.28 | 0.60 | 1.44 | -1.11 | 0.54 | False |
| ACAT1 | -0.93 | -0.72 | -1.06 | -0.96 | -0.93 | -1.15 | -0.71 | -0.47 | 0.24 | -0.25 | -0.61 | -1.13 | False |
| ACAT2 | 0.67 | 0.66 | 0.11 | 1.05 | 0.59 | -0.52 | -0.02 | -0.33 | -0.24 | 0.55 | -0.33 | 0.54 | False |
| ACMSD | 1.55 | 1.64 | 1.97 | 0.89 | 1.47 | -0.14 | -0.22 | 3.20 | 0.59 | -0.48 | -0.16 | 1.32 | False |
| AFMID | 0.63 | 0.10 | 1.06 | 0.50 | 0.47 | -0.97 | 0.50 | 0.58 | 0.52 | 0.79 | 0.32 | 0.17 | False |
| ALDH1A1 | -0.10 | -2.44 | -0.73 | -1.41 | -2.54 | 0.22 | 0.67 | -0.92 | -0.39 | 0.02 | -1.43 | -1.44 | False |
| ALDH1A2 | 0.45 | -2.77 | 1.14 | 0.57 | -0.46 | -1.69 | 0.43 | -0.80 | -1.57 | -0.11 | -1.68 | -3.39 | False |
| ALDH1B1 | -2.41 | 0.74 | 0.78 | 0.05 | 0.88 | -1.17 | -0.64 | 1.10 | -0.56 | 0.82 | -1.04 | -1.96 | False |
| ALDH2 | -1.64 | -2.10 | -0.26 | -0.17 | -0.65 | -0.75 | -1.02 | -1.19 | -0.81 | -0.23 | -0.84 | -0.36 | True |
| ALDH3A2 | -0.24 | -0.56 | -0.40 | 0.45 | -0.45 | -0.60 | 0.41 | -0.04 | -0.50 | -0.43 | 0.21 | -0.06 | False |
| ALDH7A1 | -0.42 | -0.57 | 0.17 | 0.43 | -0.04 | -0.64 | 0.22 | 0.23 | 0.05 | 0.39 | -0.78 | 0.08 | False |
| ALDH8A1 | 0.07 | 0.08 | 0.48 | 0.89 | 1.06 | -0.82 | -1.25 | -0.34 | 0.18 | 0.56 | -1.07 | 0.57 | False |
| ALDH9A1 | -0.48 | -0.26 | -0.17 | 0.17 | -0.68 | -0.67 | -0.17 | -0.18 | -0.25 | 0.06 | -0.58 | -0.35 | False |
| AOC1 | 1.79 | 3.44 | -1.70 | 1.53 | 0.86 | 0.07 | -1.16 | 4.97 | -2.10 | -0.21 | 3.05 | 6.41 | False |
| AOX1 | -3.45 | -2.17 | -1.64 | -2.08 | -0.61 | -1.33 | -0.77 | -1.20 | -2.30 | -0.44 | -2.90 | -4.96 | True |
| ASMT | -0.82 | 0.02 | 0.64 | -1.28 | 1.82 | 2.15 | 1.54 | 0.59 | 0.58 | 1.18 | -0.39 | -0.72 | False |
| CAT | -0.67 | -1.88 | -0.70 | -0.30 | 0.01 | -1.22 | -0.70 | -1.87 | -0.57 | -0.13 | -0.21 | -0.75 | False |
| CYP19A1 | -0.55 | -0.20 | 2.38 | 0.77 | 2.42 | 0.96 | 7.64 | 1.41 | -5.03 | 3.28 | -0.38 | -0.19 | False |
| CYP11A1 | 2.75 | -3.60 | 1.66 | -1.19 | -1.69 | -3.02 | 1.04 | -1.88 | 0.57 | -2.37 | -1.33 | 1.32 | False |
| CYP11A2 | 4.97 | -2.12 | 1.60 | -1.70 | 0.15 | 0.70 | -2.60 | -3.71 | -1.76 | 0.27 | -0.91 | -0.80 | False |

(Continued)

Table 2. (Continued)

| GENE NAME | BLCA | BRCA | COAD | ESCA | HNSC | KIRC | LHC | LUAD | PRAD | STAD | THCA | UCEC | FINAL |
|-----------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| CYP1B1 | -1.43 | 0.42 | -0.01 | -1.74 | 1.01 | -1.11 | 2.26 | 0.20 | 0.31 | -0.11 | 2.87 | -3.28 | False |
| CYP2A13 | -1.00 | 3.35 | 1.25 | 2.51 | -3.11 | 4.51 | 1.00 | -1.02 | -1.21 | 1.92 | 0.93 | 0.70 | False |
| CYP2C18 | -0.40 | -0.62 | -1.96 | -0.77 | -1.65 | -0.45 | -0.53 | 1.50 | 0.91 | -1.39 | -0.29 | 4.36 | False |
| CYP2E1 | -0.04 | 0.06 | 0.96 | 0.56 | -0.92 | -0.29 | -0.91 | 1.46 | -1.52 | 0.18 | 0.34 | 0.69 | False |
| CYP2F1 | -0.39 | 3.43 | 2.06 | -0.04 | -5.80 | 3.05 | 2.41 | -2.15 | -0.58 | 0.89 | 0.58 | 1.51 | False |
| CYP2J2 | 1.03 | 0.06 | -0.55 | 0.95 | -0.84 | 5.24 | -0.47 | 0.83 | 1.33 | -0.68 | 0.78 | 2.01 | False |
| CYP3A4 | 1.50 | -1.74 | -4.75 | 0.65 | -3.08 | -1.28 | -1.05 | 1.03 | 1.59 | -3.89 | -0.85 | 0.87 | False |
| CYP4F12 | 0.05 | -0.82 | -1.06 | -0.07 | -2.11 | 0.41 | -0.21 | 0.44 | -0.75 | -0.70 | -0.93 | -2.04 | False |
| CYP7B1 | -0.60 | -0.70 | 0.54 | 0.05 | -0.36 | -0.40 | 0.00 | 0.79 | -0.44 | 0.41 | -1.70 | -1.63 | False |
| DDC | 3.68 | 1.40 | -0.12 | 1.55 | 0.51 | -1.55 | 0.30 | 1.91 | 1.05 | 0.15 | -2.30 | 3.81 | False |
| DHCR24 | 1.04 | 0.90 | 0.00 | 0.17 | 0.04 | -0.63 | 0.56 | -0.99 | 0.41 | 0.10 | -0.06 | 0.23 | False |
| DHTKD1 | 0.99 | 1.04 | 0.08 | 0.67 | 0.41 | -0.60 | -0.34 | 1.06 | 0.46 | 0.82 | -0.69 | 0.85 | False |
| DLD | -0.20 | -0.23 | -0.19 | 0.40 | 0.06 | -0.88 | 0.43 | 0.12 | -0.12 | 0.59 | -0.40 | -0.12 | False |
| DLST | -0.28 | -0.45 | -0.28 | 0.43 | 0.61 | -0.87 | 0.35 | 0.00 | -0.14 | 0.21 | -0.03 | -0.49 | False |
| ECHS1 | 0.47 | -0.32 | -0.30 | 0.40 | -0.13 | -1.25 | -0.53 | 0.50 | 0.26 | 0.34 | -0.15 | 0.88 | False |
| EHHADH | 0.45 | -0.96 | -0.99 | 0.21 | 0.82 | -0.56 | -0.61 | 0.20 | 0.29 | 0.48 | -0.71 | -0.28 | False |
| GCDH | 0.26 | 0.19 | 0.39 | 0.38 | 0.17 | -0.98 | -0.64 | 0.25 | 0.31 | 0.26 | -0.51 | 0.42 | False |
| HAAO | -2.27 | -0.89 | -0.04 | 0.28 | -0.28 | 0.49 | -0.71 | -0.48 | -1.11 | -0.11 | -0.27 | -1.86 | False |
| HADH | 0.04 | -1.17 | -0.72 | -0.27 | -0.54 | -1.70 | -0.13 | -0.01 | -0.01 | -0.48 | -0.31 | -0.58 | False |
| HADHA | -0.35 | -0.54 | -0.73 | 0.16 | 0.02 | -0.13 | 0.28 | -0.17 | -0.07 | -0.17 | -0.16 | -0.34 | False |
| HSD17B10 | 0.42 | 0.87 | 1.02 | 0.94 | 0.21 | -0.52 | 0.13 | 0.52 | 0.44 | 0.58 | -0.50 | 0.98 | False |
| IDO1 | 2.19 | 2.10 | 0.78 | 3.16 | 1.56 | 3.39 | 1.56 | 0.36 | 0.39 | 4.15 | -1.14 | 2.43 | False |

(Continued)

Table 2. (Continued)

| GENE NAME | BLCA | BRCA | COAD | ESCA | HNSC | KIRC | LIHC | LUAD | PRAD | STAD | THCA | UCEC | FINAL |
|-----------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| IDO2 | -0.33 | 1.68 | -0.07 | 1.16 | 1.57 | -0.01 | -1.20 | 1.08 | 1.70 | 1.71 | -2.16 | 4.46 | False |
| IL4I1 | 1.09 | 2.63 | 1.13 | 2.24 | 1.88 | 2.14 | 2.33 | 1.96 | 0.77 | 1.93 | 0.41 | 0.97 | False |
| INMT | -2.13 | -2.43 | -0.13 | -1.18 | -1.12 | -0.27 | -1.83 | -3.44 | -1.17 | -0.68 | 1.83 | -4.07 | False |
| KMO | 1.32 | 1.66 | -0.28 | 1.31 | 2.80 | 0.46 | -1.17 | -1.24 | 0.32 | 0.99 | -0.31 | 0.59 | False |
| KYAT1 | -0.36 | 0.01 | 1.31 | 0.72 | 0.68 | 0.11 | 1.16 | 0.39 | -0.05 | 0.47 | -0.11 | -0.34 | False |
| KYAT3 | -0.07 | -0.17 | 0.35 | -0.01 | 0.20 | -0.64 | 0.48 | 0.02 | 0.12 | 0.52 | 0.09 | -0.45 | False |
| KYNU | 1.01 | 0.43 | 1.27 | 2.82 | 2.42 | -0.10 | -0.01 | 1.17 | -0.24 | 1.32 | -0.24 | 1.00 | False |
| MAOA | -0.45 | -3.15 | -1.75 | -0.69 | -0.17 | -0.72 | 0.08 | -0.81 | 0.59 | -0.92 | -0.03 | -1.66 | False |
| MAOB | -3.06 | -0.72 | -2.08 | -0.57 | -1.30 | 0.57 | -0.18 | -1.49 | -0.90 | -1.17 | -0.74 | -3.61 | False |
| MDM2 | 0.83 | 0.41 | 0.77 | 1.03 | 0.33 | 0.90 | 0.52 | 0.21 | -0.07 | 1.65 | 0.54 | 0.23 | False |
| OGDH | -0.45 | -0.17 | -0.41 | 0.27 | 0.04 | -0.50 | 0.96 | -0.41 | -0.24 | 0.05 | -0.53 | -0.25 | False |
| PRMT1 | 0.55 | 0.57 | 1.42 | 1.47 | 0.67 | 0.36 | 1.32 | 0.40 | 0.09 | 0.93 | 0.09 | 0.50 | False |
| RNF25 | 0.00 | 0.32 | 0.45 | 0.78 | 0.63 | 0.26 | 1.00 | 0.23 | 0.17 | 0.39 | 0.01 | 0.20 | False |
| TDO2 | 2.32 | 3.76 | 2.18 | 4.33 | 2.79 | 2.10 | -1.16 | 1.72 | 1.57 | 1.40 | 1.32 | 2.55 | False |
| TPH1 | -0.30 | 1.90 | -3.37 | 1.30 | 0.32 | 0.61 | 2.73 | 0.35 | -0.65 | 1.21 | 0.87 | 2.65 | False |
| TPH2 | 1.39 | 2.38 | 0.41 | 0.46 | 0.95 | 2.36 | 0.34 | 2.91 | 0.58 | 1.91 | -2.29 | 1.59 | False |
| UBE3A | -0.30 | 0.08 | 0.09 | 0.70 | 0.29 | -0.04 | 0.41 | -0.04 | -0.09 | 0.75 | -0.37 | -0.71 | False |
| UBR5 | 0.17 | 0.19 | 0.61 | 0.92 | 1.15 | -0.26 | 1.43 | 0.58 | 0.09 | 1.08 | -0.33 | -0.30 | False |
| WARS1 | 1.45 | 0.42 | 0.58 | 1.85 | 2.53 | 0.36 | 1.00 | -0.27 | 0.19 | 2.02 | 0.59 | 0.62 | False |

Table 3. Positively correlated gene pairs which are conserved in all 12 types of cancer.

| <i>ACAT2-INMT</i> | <i>DLST-HADH</i> | <i>MDM2-UBE3A</i> |
|----------------------|-----------------------|-------------------|
| <i>CAT-EHHADH</i> | <i>GCDH-UBR5</i> | <i>UBE3A-UBR5</i> |
| <i>CAT-HADH</i> | <i>HSD17B10-RNF25</i> | <i>DLD-DLST</i> |
| <i>DHTKD1-EHHADH</i> | <i>IL4I1-WARS1</i> | <i>HADH-HADHA</i> |

types of cancer (Table 3). PPI network was plotted using Cytoscape (<http://www.cytoscape.org>).²⁸

Results and Discussion

RNAseq datasets and matching clinical sample sheets were extracted for 12 types of cancer from The Cancer Genome Atlas (TCGA-BLCA, TCGA-BRCA, TCGA-COAD, TCGA-ESCA, TCGA-HNSC, TCGA-KIRC, TCGA-LIHC, TCGA-LUAD, TCGA-PRAD, TCGA-STAD, TCGA-THCA, and TCGA-UCEC). Expression of 62 genes involved in tryptophan metabolism was extracted from the DEG list (Table 2). Only gene *AOX1* was downregulated in all the 12 types of cancer, whereas the other genes exhibited variant patterns of alternation in expression. This implicates that tissue- and cell-specific heterogeneity exists for the tryptophan metabolic pathway in carcinogenesis. The current result was consistent with previous reports of differential interplay of oncogenic and metabolic signaling pathways.^{29,30} It was noteworthy that several genes were significantly up- or down-regulated ($|\log_2FC| > 2$) across some tumor types (Table 2), and thus could be potential biomarkers and/or drug-design targets for these tumor types. For example, *AOX1* (−3.45), *MAOB* (−3.06), *INMT* (−2.13), *HAAO* (−2.27), and *ALDH1B1* (−2.41) were downregulated and *CYP1A1* (2.75), *CYP1A2* (4.97), *DDC* (3.68), *IDO1* (2.19), and *TDO2* (2.32) were upregulated in TCGA-BLCA. However, gene expression is not binary and may vary over a broad range for multifactorial diseases such as cancer. Clinical heterogeneity, etiology, cancer subtypes and stage of the disease can affect respective gene expression.³¹ It should be cautious to consider these genes as potential biomarkers. Nevertheless, the DEG analysis provided valuable information on how tryptophan metabolism is involved in carcinogenesis and cancer progression.

As the only gene downregulated across all 12 types of cancer, *AOX1* encodes aldehyde oxidase 1 (AOX1), a flavin-containing monooxygenase, which detoxifies some aldehydes and nitrogenous heterocyclic compounds, specifically sulfur- and nitrogen-containing xenobiotics.^{32,33} A discrepancy has been observed on the role AOX1 plays in cancer. AOX1 was reported to inhibit breast cancer development,³⁴ but promote prostate cancer proliferation.³⁵ Gene *AOX1* was epigenetically silenced during bladder cancer progression and downregulated in colon cancer.^{33,36} Furthermore, *AOX1* was proposed as a downregulated pan-cancer biomarker candidate based on

transcriptome analysis of both FANTOM5 cancer cell lines and TCGA primary tumors.³⁷ However, any physiological or pathophysiological process requires a delicate coordination of multiple genes. In this study, we used tryptophan metabolism as an example to illustrate that gene coordination and homeostasis were disrupted *via* decoupling (loss of correlation) or coupling (gain of correlation) of gene expressions during carcinogenesis.³⁸

Loss or gain of gene pair correlations inadvertently affects the dynamically modulated interaction between cellular physiology and environment. It has been reported that certain genes become pair-correlated during cancer progression.^{24,25,39,40} Herein, we investigated whether there is any pattern change in gene pair correlation coefficient for the 62 genes in tryptophan metabolism pathway across the 12 types of cancer. Pairwise gene correlation coefficients were calculated for both normal controls and cancer patients. As shown in Figure 1, genes in the tryptophan metabolic pathway were widely and strongly correlated in normal controls but showed a significant loss of pairwise correlation in tumor patients for all 12 types of cancer. Twelve pairs of genes were observed to be positively coupled in all 12 types of cancer (Table 3). They were *ACAT2-INMT*, *DLST-HADH*, *MDM2-UBE3A*, *CAT-EHHADH*, *GCDH-UBR5*, *UBE3A-UBR5*, *CAT-HADH*, *HSD17B10-RNF25*, *DLD-DLST*, *DHTKD1-EHHADH*, *IL4I1-WARS1*, and *HADH-HADHA*. We mapped these genes into the tryptophan metabolic pathway (Figure 2). Seven genes, *ACAT2*, *HADHA*, *EHHADH*, *HADH*, *DLD*, *DLST*, and *DHTKD1*, affect the switch of metabolism towards glycolysis in cancer cells. Increased aerobic glycolysis has been recognized as a characteristic phenomenon in cancer. *WARS1* encodes tryptophanyl-tRNA synthetase 1 (WARS1), which influences cancer cell proliferation and oncogenic transformation.⁴¹ *IL4I1* encodes interleukin 4 induced protein 1 (IL4I1), an immunosuppressive enzyme. It is overexpressed in tumor cells and activates aryl hydrocarbon receptor by tryptophan catabolites, thereby promoting tumor progression and suppressing immune response towards cancer.⁴² *IL4I1* also promotes cancer cell motility (<https://www.genengnews.com/news/checkpoint-molecule-could-be-new-cancer-immunotherapy-target/>). Five genes, *MDM2*, *UBR3*, *UBE3A*, *RNF25*, and *UBR5*, were involved in ubiquitination. Ubiquitin-proteasome system (UPS) is critical in maintaining protein homeostasis *via* eliminating damaged, misfolded and unnecessary proteins. Interestingly, 4 out of the 5 genes encode E3 ligases, which are key drug-design targets.⁴³ Furthermore, we generated the PPI network for these genes (Figure 3). It was clearly observed that most of the proteins encoded by these genes, such as MDM2, DLD, HSD17B10, UBE3A, HADHA, and DLST, were highly likely to be biological hubs that modulate important biological and physiological functions. Therefore, these hub proteins are potential targets in developing novel anticancer agents. We hypothesize that therapeutic agents that disrupt the positive correlations among the 12 pairs of genes would reduce tumor

progression and be beneficial for cancer patients. The proteins encoded by these genes are listed in Table 4.

Gain of correlation for the 12 pairs of genes suggests 2 potential applications. First, follow-up of gene pair correlation coefficients for the tryptophan metabolic pathway could be a valid approach for early detection of cancer. Since cancer is usually associated with chronic inflammation and about 25% of cancer is even caused by infection and inflammation,^{44,45} the current approach may also be applied for early detection of pre-cancerous inflammation. Secondly, population-based screening

of genes in tryptophan metabolism could provide a glimpse of cancer distribution within a community. Nonetheless, the current study supports the important role of tryptophan metabolism in cancer and offers a systematic and novel method to use gene pair correlation coefficients as a potential biomarker of cancer. Have to also consider the relationship between age and tryptophan metabolism. In breast cancer, most advanced/aggressive breast cancer subtypes occurred in younger women while less aggressive in older women. Kynurenine metabolism is also influenced by age.^{46–48}

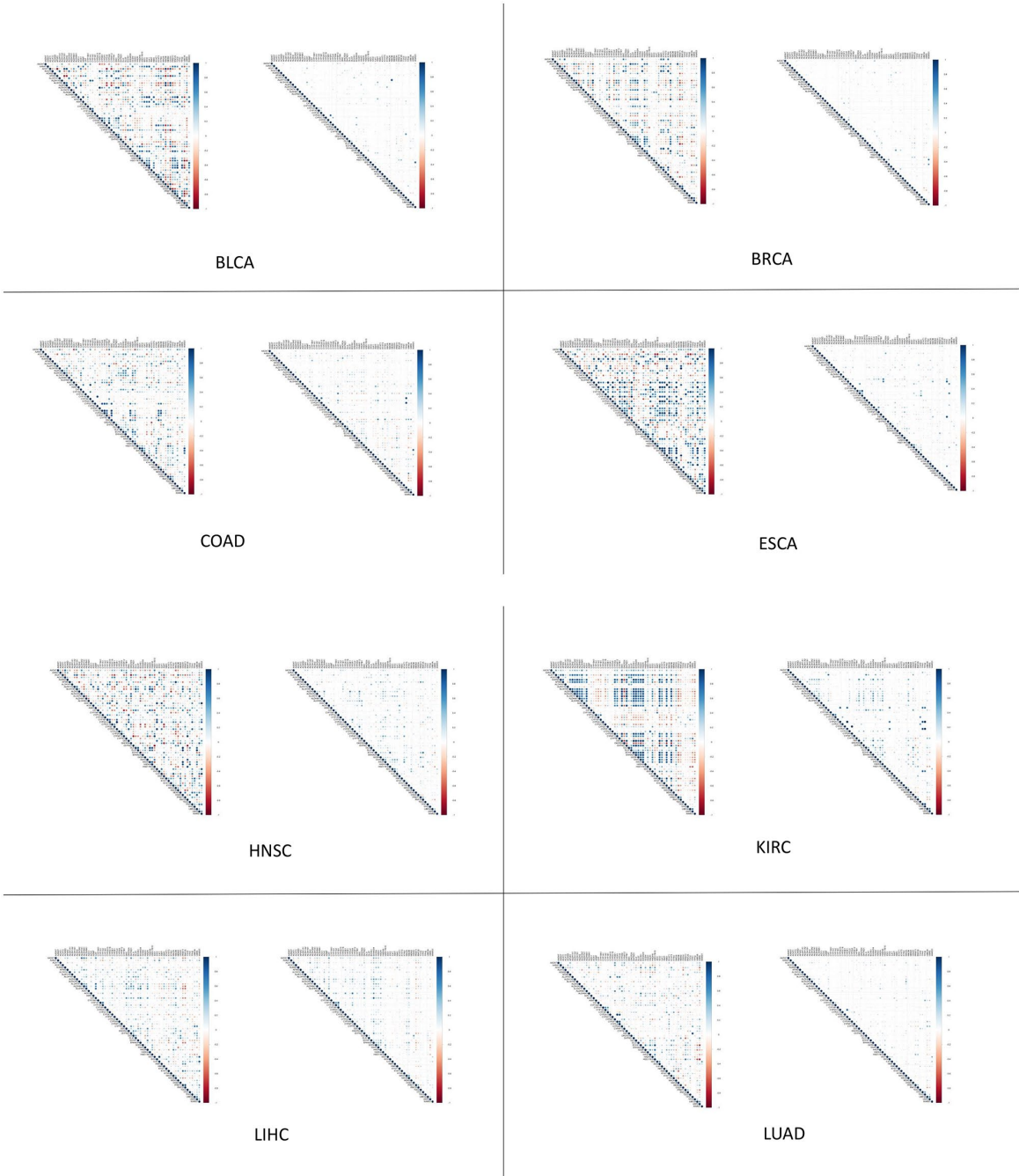


Figure 1. (Continued)

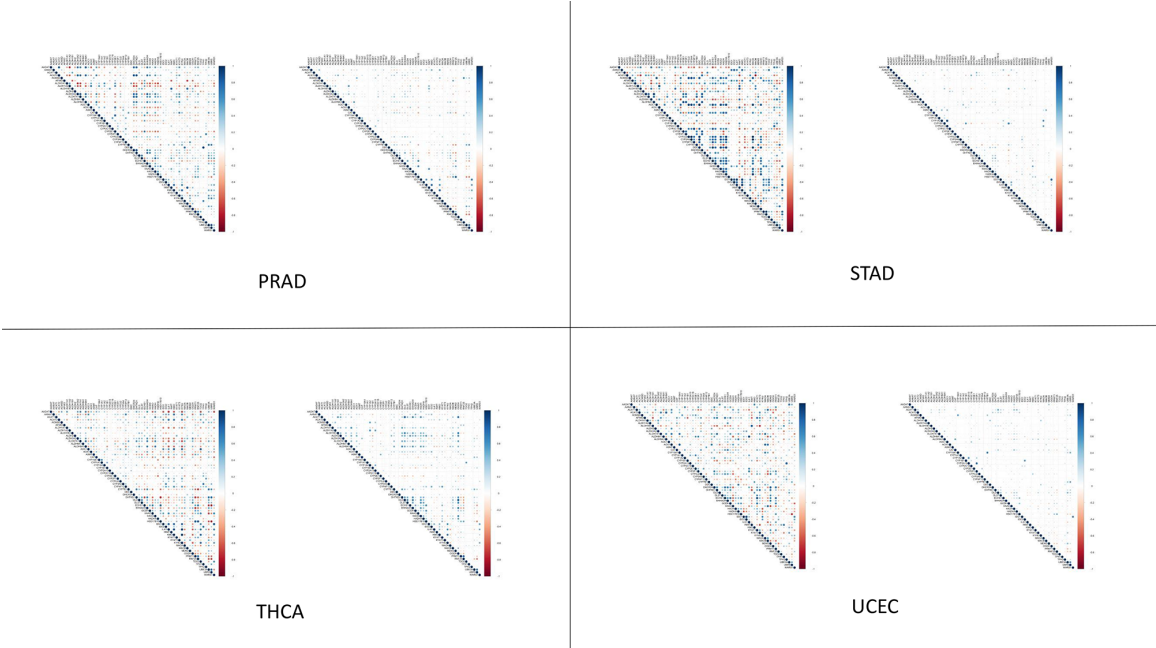


Figure 1. Pairwise correlations of 62 genes involved in tryptophan metabolism in normal controls (left) and tumor patients (right) across 12 types of cancer. Positive and negative correlations were represented in blue and red dots, respectively, and the sizes of the dots are proportional to the correlation values.

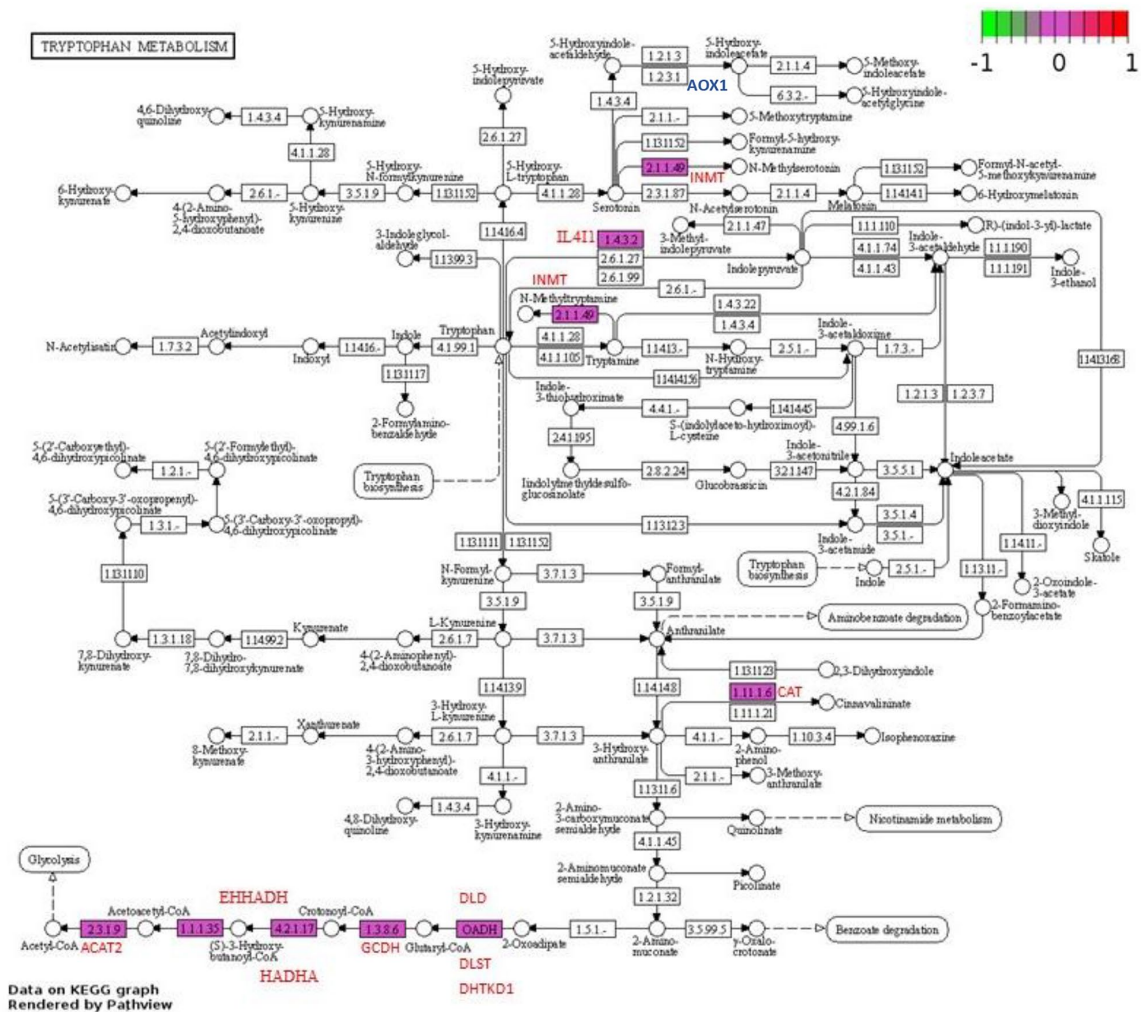


Figure 2. Mapping the 12 pairs of genes, which are positively correlated in all 12 types of cancer, into the tryptophan metabolic pathway.

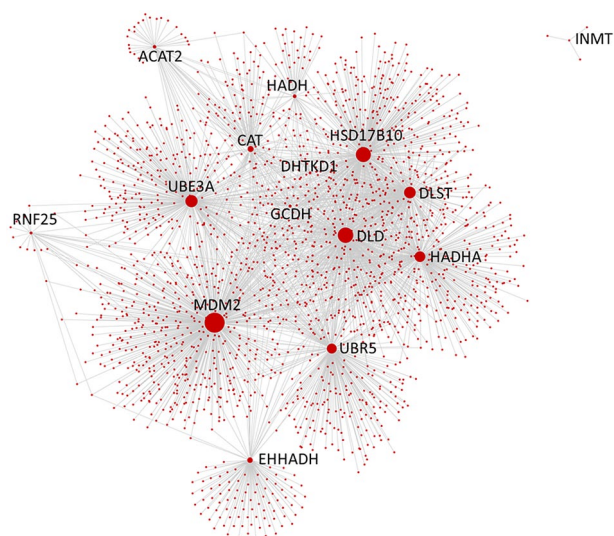


Figure 3. Protein-protein interaction (PPI) network for the 12 pairs of genes that were positively correlated across 12 types of cancer.

Table 4. Proteins encoded by genes that were observed to be positively coupled in all 12 types of cancers.

| GENE SYMBOL | NAME OF ENCODED PROTEIN |
|-------------|---|
| ACAT2 | Cytosolic acetoacetyl-CoA thiolase |
| INMT | N-methylates indoles such as tryptamine |
| HADH | Hydroxyacyl-coenzyme A dehydrogenase |
| MDM2 | E3 ubiquitin-protein ligase Mdm2 |
| CAT | Catalase |
| EHHADH | Enoyl-CoA hydratase and 3-hydroxyacyl CoA dehydrogenase |
| GCDH | Glutaryl-CoA dehydrogenase |
| UBR5 | Ubiquitin-protein ligase E3 component N-recognin 5 |
| UBE3A | Ubiquitin-protein ligase E3A |
| HSD17B10 | 3-Hydroxyacyl-CoA dehydrogenase type II |
| RNF25 | E3 ubiquitin-protein ligase RNF25 |
| DLD | Dihydrolipoamide dehydrogenase |
| DLST | Dihydrolipoamide S-succinyltransferase |
| DHTKD1 | Dehydrogenase E1 and transketolase domain containing 1 |
| IL4I1 | Interleukin 4 induced 1 |
| WARS1 | Tryptophanyl-tRNA synthetase 1 |
| HADHA | Hydroxyacyl-CoA dehydrogenase trifunctional multienzyme complex subunit alpha |

Conclusion

In this study, we investigated the expression and pairwise correlation of 62 genes involved in tryptophan metabolism. Out of the 62 genes, only gene *AOX1*, encoding aldehyde oxidase 1, was ubiquitously downregulated. Gene pair correlations were

extensive and strong in normal controls but dramatically decreased/lost in tumor patients for all 12 types of cancer. Only 12 pairs of genes were still positively correlated in cancer. The current results suggested that gene pair correlations of the tryptophan metabolic pathway could be a prognostic and/or diagnostic biomarker for cancer.

Author Contributions


Conceptualization, MSK and JY; methodology and software, MSK, JY and SKD; formal analysis, MSK, JY, GJG and SKD; investigation, MSK, JY and KR; writing-original draft preparation, MSK, JY, and GJG; writing-review and editing, JY, GJG, BH, NB and MSK; project administration, MKS and JY. All authors have read and agreed to the published version of the manuscript.

ORCID iDs

Meena Kishore Sakharkar  <https://orcid.org/0000-0002-9289-3314>

Benjamin Heng  <https://orcid.org/0000-0002-0247-2920>

Nady Braidy  <https://orcid.org/0000-0002-0497-5572>

Gilles J Guillemin  <https://orcid.org/0000-0001-8105-4470>

REFERENCES

1. Zaorsky NG, Churilla TM, Egleston BL, et al. Causes of death among cancer patients. *Ann Oncol.* 2017;28:400–407.
2. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. *CA Cancer J Clin.* 2020;70:7–30.
3. Hammoudi N, Ahmed KB, Garcia-Prieto C, Huang P. Metabolic alterations in cancer cells and therapeutic implications. *Chin J Cancer.* 2011;30:508–525.
4. Yoshida GJ. Metabolic reprogramming: the emerging concept and associated therapeutic strategies. *J Exp Clin Cancer Res.* 2015;34:111.
5. Pavlova NN, Thompson CB. The emerging hallmarks of cancer metabolism. *Cell Metab.* 2016;23:27–47.
6. Zhang Y, Zhang Z. The history and advances in cancer immunotherapy: understanding the characteristics of tumor-infiltrating immune cells and their therapeutic implications. *Cell Mol Immunol.* 2020;17:807–821.
7. Lukey MJ, Katt WP, Cerione RA. Targeting amino acid metabolism for cancer therapy. *Drug Discov Today.* 2017;22:796–804.
8. Opitz CA, Somarrivas Patterson LF, Mohapatra SR, et al. The therapeutic potential of targeting tryptophan catabolism in cancer. *Br J Cancer.* 2020;122:30–44.
9. Günther J, Fallarino F, Fuchs D, Wirthgen E. Immunomodulatory roles of tryptophan metabolites in inflammation and cancer. *Front Immunol.* 2020;11:1497.
10. Vacchelli E, Aranda F, Eggermont A, et al. Trial watch: IDO inhibitors in cancer therapy. *Oncotarget.* 2014;3:e957994.
11. Liu M, Wang X, Wang L, et al. Targeting the IDO1 pathway in cancer: from bench to bedside. *J Hematol Oncol.* 2018;11:100.
12. Fallarino F, Grohmann U, Vacca C, et al. T cell apoptosis by tryptophan catabolism. *Cell Death Differ.* 2002;9:1069–1077.
13. Terness P, Bauer TM, Röse L, et al. Inhibition of allogeneic T cell proliferation by indoleamine 2,3-dioxygenase-expressing dendritic cells: mediation of suppression by tryptophan metabolites. *J Exp Med.* 2002;196:447–457.
14. Munn DH, Sharma MD, Baban B, et al. GCN2 kinase in T cells mediates proliferative arrest and anergy induction in response to indoleamine 2,3-dioxygenase. *Immunity.* 2005;22:633–642.
15. Della Chiesa M, Carlomagno S, Frumento G, et al. The tryptophan catabolite L-kynurenine inhibits the surface expression of Nkp46- and NKG2D-activating receptors and regulates NK-cell function. *Blood.* 2006;108:4118–4125.
16. Wang Q, Liu D, Song P, Zou MH. Tryptophan-kynurenine pathway is dysregulated in inflammation, and immune activation. *Front Biosci (Landmark Ed).* 2015;20:1116–1143.
17. Mellor AL, Chandler P, Baban B, et al. Specific subsets of murine dendritic cells acquire potent T cell regulatory functions following CTLA4-mediated induction of indoleamine 2,3 dioxygenase. *Int Immunol.* 2004;16:1391–1401.
18. Smith C, Chang MY, Parker KH, et al. IDO is a nodal pathogenic driver of lung cancer and metastasis development. *Cancer Discov.* 2012;2:722–735.

19. Sforzini L, Netti MA, Mondelli V, et al. Inflammation in cancer and depression: a starring role for the kynurenine pathway. *Psychopharmacology*. 2019;236:2997–3011.
20. Hornyak L, Dobos N, Koncz G, et al. The role of indoleamine-2,3-dioxygenase in cancer development, diagnostics, and therapy. *Front Immunol*. 2018;9:151.
21. Chuang SC, Fanidi A, Ueland PM, et al. Circulating biomarkers of tryptophan and the kynurenine pathway and lung cancer risk. *Cancer Epidemiol Biomarkers Prev*. 2014;23:461–468.
22. Brandacher G, Perathoner A, Ladurner R, et al. Prognostic value of indoleamine 2,3-dioxygenase expression in colorectal cancer: effect on tumor-infiltrating T cells. *Clin Cancer Res*. 2006;12:1144–1151.
23. Weinlich G, Murr C, Richardsen L, et al. Decreased serum tryptophan concentration predicts poor prognosis in malignant melanoma patients. *Dermatology*. 2007;214:8–14.
24. Magen A, Das Sahu A, Lee JS, et al. Beyond synthetic lethality: charting the landscape of pairwise gene expression states associated with survival in cancer. *Cell Rep*. 2019;28:938–948.
25. Ling B, Chen L, Liu Q, et al. Gene expression correlation for cancer diagnosis: a pilot study. *Biomed Res Int*. 2014;2014:e253804.
26. R. The R project for statistical computing. 2020. Updated 2020. Accessed on May 15, 2020. <https://www.r-project.org>
27. Chatr-Aryamontri A, Oughtred R, Boucher L, et al. The BioGRID interaction database: 2017 update. *Nucleic Acids Res*. 2017;45:D369–D379.
28. Shannon P, Markiel A, Ozier O, et al. Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Res*. 2003;13:2498–2504.
29. Schneider G, Schmidt-Supprian M, Rad R, et al. Tissue-specific tumorigenesis: context matters. *Nat Rev Cancer*. 2017;17:239–253.
30. Lyssiotis CA, Kimmelman AC. Metabolic interactions in the tumor microenvironment. *Trends Cell Biol*. 2017;27:863–875.
31. Sun XX, Yu Q. Intra-tumor heterogeneity of cancer cells and its implications for cancer treatment. *Acta Pharmacol Sin*. 2015;36:1219–1227.
32. Maeda K, Ohno T, Igarashi S, et al. Aldehyde oxidase 1 gene is regulated by Nrf2 pathway. *Gene*. 2012;505:374–378.
33. Zhang W, Chai W, Zhu Z, et al. Aldehyde oxidase 1 promoted the occurrence and development of colorectal cancer by up-regulation of expression of CD133. *Int Immunopharmacol*. 2020;85:106618.
34. Li W, Middha M, Bicak M, et al. Genome-wide scan identifies role for AOX1 in prostate cancer survival. *Eur Urol*. 2018;74:710–719.
35. Singh B, Shoulson R, Chatterjee A, et al. Resveratrol inhibits estrogen-induced breast carcinogenesis through induction of NRF2-mediated protective pathways. *Carcinogenesis*. 2014;35:1872–1880.
36. Vantaku V, Putluri V, Bader DA, et al. Epigenetic loss of AOX1 expression via EZH2 leads to metabolic deregulations and promotes bladder cancer progression. *Oncogene*. 2020;39:6387–6392.
37. Kaczowski B, Tanaka Y, Kawaji H, et al. Transcriptome analysis of recurrently deregulated genes across multiple cancers identifies new pan-cancer biomarkers. *Cancer Res*. 2016;76:216–226.
38. Sakharkar MK, Kaur Dhillon S, Chidambaram SB, et al. Gene pair correlation coefficients in sphingolipid metabolic pathway as a potential prognostic biomarker for breast cancer. *Cancers (Basel)*. 2020;12:1747.
39. Naderi A. SRARP and HSPB7 are epigenetically regulated gene pairs that function as tumor suppressors and predict clinical outcome in malignancies. *Mol Oncol*. 2018;12:724–755.
40. Park B, Lee W, Park I, et al. Finding prognostic gene pairs for cancer from patient-specific gene networks. *BMC Med Genom*. 2019;12:e179.
41. Adam I, Dewi DL, Mooiweer J, et al. Upregulation of tryptophanyl-tRNA synthetase adapts human cancer cells to nutritional stress caused by tryptophan degradation. *Oncimmunology*. 2018;7:e1486353.
42. Sadik A, Somarribas-Patterson LF, Öztürk S, et al. IL4I1 is a metabolic immune checkpoint that activates the AHR and promotes tumor progression. *Cell*. 2020;182:1252–1270.
43. Shi D, Grossman SR. Ubiquitin becomes ubiquitous in cancer: emerging roles of ubiquitin ligases and deubiquitinases in tumorigenesis and as therapeutic targets. *Cancer Biol Ther*. 2010;10:737–747.
44. Coussens LM, Werb Z. Inflammation and cancer. *Nature*. 2002;420:860–867.
45. Murata M. Inflammation and cancer. *Environ Health Prev Med*. 2018;23:50.
46. Refaey ME, McGee-Lawrence ME, Fulzele S, et al. Kynurenine, a tryptophan metabolite that accumulates with age, induces bone loss. *J Bone Miner Res*. 2017;32:2182–2193.
47. Kim BJ, Hamrick MW, Yoo HJ, et al. The detrimental effects of kynurenine, a tryptophan metabolite, on human bone metabolism. *J Clin Endocrinol Metab*. 2019;104:2334–2342.
48. de Bie J, Guest J, Guillemin GJ, Grant R. Central kynurenine pathway shift with age in women. *J Neurochem*. 2016;136:995–1003.