Introduction

Cancer is the second most-frequent cause of death worldwide, killing more than 8 million people each year. According to Cancer Statistics 2020, it was estimated that there would be approximately 1,806,590 new cases in 2020. 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. Reprogramming of cellular metabolism has been observed in cancer cells to maintain viability and building biomass. Furthermore, cancer cells can express abnormal antigens to evade host immunosurveillance. 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. Tryptophan is an essential amino acid, and its catabolism is involved in both immunomodulation and cancer development.

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. IDO1 overexpression causes a decrease of tryptophan and an accumulation of kynurenine in local tumour environment, leading to suppressed immune function T-cell and natural killer cells. Activation of IDO1 also leads to the production of regulatory T cells and myeloid derived suppressor cells. 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. Furthermore, survival time was observed to be shorter for cancer patients with a low serum level of tryptophan.

Metabolism as a Hallmark in Cancer Diagnosis

Altering the expression of IDO1 to more efficiently inhibit its activity could potentially be a useful treatment strategy, and tumor infiltrating lymphocytes (TILs) have been shown to have suppressive effects on IDO1. Reprogramming of cellular metabolism has been observed in cancer cells to maintain viability and building biomass. Furthermore, cancer cells can express abnormal antigens to evade host immunosurveillance. 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. Tryptophan is an essential amino acid, and its catabolism is involved in both immunomodulation and cancer development.

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

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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. Our studies also showed that gene expression correlation coefficients could be used as a prognosis biomarker for human breast cancer. 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.gene_cards.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 60,483 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. Likelihood Ratio Test (LRT) was applied, and the sample expression profiles were screened using \( P \text{-value} < .001 \). The output was expressed in normalized log2-fold-change (Log2FC). 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). 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 | LIHC | 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 |
| CYP1A1    | 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 |
| CYP1A2    | 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)
| GENE NAME | BLCA | BRCA | COAD | ESCA | HNSC | KIRC | LIHC | 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 | −0.92| −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| −0.48| −0.31| −0.16| −0.34| 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)
| 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 |
types of cancer (Table 3). PPI network was plotted using Cytoscape (http://www.cytoscape.org).28

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

| Gene 1 | Gene 2 | Gene 3 |
|-------|-------|-------|
| ACAT2-INMT | DLST-HADH | MDM2-UBE3A |
| CAT-EHHADH | GCDH-UBR5 | UBE3A-UBR5 |
| CAT-HADH | HSD17B10-RNF25 | DLD-DLST |
| DHTKD1-EHHADH | IL4I1-WARS1 | HADH-HADHA |

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\(\text{FC}\)| > 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, the current approach may also be applied for early detection of precancerous 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.
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.
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.

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**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.

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

| GENE SYMBOL | NAME OF ENCODED PROTEIN |
|-------------|-------------------------|
| ACAT2       | Cytosolic acetoacetyl-CoA thiolase |
| INMT        | N-methyltetrahydroindole such as tryptamine |
| HADH        | Hydroxacyl-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-succinylltransferase |
| DHTKD1      | Dihydrolipoamide E2 containing 1 and transketolase domain containing 1 |
| IL4I1       | Interleukin 4 induced 1 |
| WARS1       | Tryptophanyl-tRNA synthetase 1 |
| HADHA       | Hydroxacyl-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
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