lncRNA EGOT across cancers: TCGA analysis

Keywords
oncogene, biomarker, lncRNA, TCGA, suppressor, non-coding RNA, viruses-depending oncogenesis

Abstract

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Material and methods
EGOT was analyzed based on the TCGA, including pathological and clinical features, cellular pathways, and genomic and cellular changes.

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Conclusions: EGOT seems to be a potential prognostic biomarker in clinical use. One of the possibilities that connected all of the analyzed types of cancers and changes in *EGOT* expression is viral activity and immunological response to viral infection.

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1. Introduction

Long non-coding RNA (lncRNA) molecules are defined as non-protein-coding RNAs longer than 200 nucleotides [1,2]. They play diverse regulatory roles depending on their cellular localization. In the nucleus, lncRNAs participate in chromatin remodeling and RNA processing, whereas in the cytoplasm, they are involved in maintaining mRNA stability, regulating translation, and modulating signaling cascades [1-6]. lncRNAs are recognized as key regulators of cellular states, and alterations in their expression and function have been linked to various pathologies, including cancer. Additionally, they hold potential as prognostic

markers and indicators of sensitivity to chemo- and radiotherapy, and could be used as potential targets for treatment personalization using nano-delivery systems [1-9].

One of the analyzed lncRNA is the eosinophil granule ontogeny transcript (*EGOT*). The human *EGOT* gene is located on the antisense strand of the intron of the inositol 1,4,5-trisphosphate receptor type 1 (*ITPR1*) gene. It should be noted that the *EGOT* transcript has two known splicing variants with the same transcriptional start site, both polyadenylated. However, they have different lengths, and a longer variant, known as *EGO-A* is unspliced, and a shortened version, *EGO-B*, is the spliced version. *EGOT* functions as a non-coding RNA in eosinophil cells development by regulation of specific eosinophil granule proteins [10]. Our previously published analysis of the *EGOT* based on the TCGA indicated that expression levels of this lncRNA were slightly down-regulated. Moreover, *EGOT* expression varies according to age, N-stage, grade and lymph node dissection. Moreover, its expression levels were helpful for the assessment of patients' survival, and *EGOT* lncRNA seems to be a prognostic biomarker. It should be emphasized that *EGOT* was also associated with genes connected with cell division, proliferation, protein modification, drug response and cell motility [6]. Numerous recent, mostly *in vitro* studies have been dedicated to understanding the role of *EGOT* in breast cancer [11-15], gastric cancer [16], renal cell carcinoma [17], glioma [18], rectal cancer [19], colon cancer [20], thyroid cancer [21], papillary thyroid carcinoma [22], laryngeal squamous cell carcinoma [23], cirrhotic hepatocellular carcinoma [24] and in non-cancerous cells such as renal tubular cells where its regulates hypoxia-induced autophagy [25] and EGOT takes role in the attenuate hypoxia-induced injury of cardiomyocytes [26], immune processes in periodontal tissues [27], as well as in regulation of the process of heart failure [28]. Moreover, it was indicated that *EGOT* was associated with viral infections, including those with oncogenic properties such as human papillomavirus (HPV) in HNSCC and hepatitis C virus (HCV) [29,30] as well as in the case of infection by SARS-CoV-2 virus [31].

In this study, we based on the TCGA project and the RNA expression databases portals [35] analyzed 27 types of cancers and verified available data about *EGOT* in the context of its biological role, association with viral-induced tumorigenesis and potential diagnostic utility.

2. Materials and methods

2.1. Patients

We used the available patient datasets of the TCGA cancers and analyzed them using the cBioPortal, GEPIA2, the UALCAN, the ENCORI databases [34]. In the case of breast

invasive carcinoma (BRCA), there were 1,072 female and 12 male patients; for head and neck squamous cell carcinoma (HNSC), 141 female and 382 male patients; for kidney renal clear cell carcinoma (KIRC), 186 female and 326 male patients; and for liver hepatocellular carcinoma (LIHC), 121 female and 251 male patients were included with the median ages of 58, 61, 60 and 61 years at the time of diagnosis, respectively. The analyzed groups consisted mostly of white race patients, with 69.3% for BRCA, 85.7% for HNSC, 86.3% for KIRC and 49.2% for LIHC, of these, those identified as ethnically non-Hispanic or Latino constituted 80.9%, 88.0%, 66.8% and 90.3% of the respective cancer groups. In terms of tumor subtype for BRCA, the following distribution were observed: luminal A with 46.0%, luminal B with 18.2%, basal with 15.8%, Her2 with 7.2%, normal with 3.3%, and 9.5% of patients not analyzed; for HNSC: 79.3% were human papillomavirus (HPV) positive, 13.8% were HPV negative and 6.9% had an undefined HPV status; for KIRC and LIHC, 68.8% and 93.5% were classified as kidney renal clear cell carcinoma and liver hepatocellular carcinoma, respectively. All data are presented in Figure 1. The number of patients included in the analyses is summarized in Table 1 and in Table 2, along with the databases used.

Next, we focused on the expression levels of *EGOT* in BRCA, HNSC, KIRC and in LIHC, utilizing the TCGA PanCancer Atlas data: mRNA expression z-scores threshold ± 2 relative to diploid samples (RNA Seq V2 RSEM), downloaded from cBioPortal. For the analysis of immune subtype, TCGA subtype, cellular features such as proliferation, wound healing ability, stromal fraction and intratumor heterogeneity we applied supplementary materials published by Thorsson et al. [36].

2.2. Survival analyses

We used the OS (overall survival) and RFS (relapse-free survival) survival parameters as the main clinical feature for selection of only those types of cancers where *EGOT* could have potential diagnostic and biological effects. We applied the GEPIA2 (Gene Expression Profiling Interactive Analysis, version 2.0) portal and patients were divided into two groups with *EGOT*-low and *EGOT*-high expression levels based on the median expression measured within the whole specific type of cancer cohort. Next Log-Rank (Mantel–Cox) tests with 95% confidence interval (CI) were applied. We took for further analysis only those of types of cancer where $p < 0.05$ was achieved.

2.3. *Analysis of molecular pathways and biological processes*

For the assessment of cellular phenotype, which is connected with the *EGOT* we used data obtained from cBioPortal. Due to the need for a deeper analysis of all pathways, the cut-off point R < -0.1 and R > 0.1 was selected for the Spearman correlation, which allows detecting even subtle trends in data and allows for indicating the enhancement or weakening of a given pathway or cellular process. Obtained gene lists were then analyzed using REACTOME pathway browser with p-value ≤ 0.05 as cut off value, similarly as described previously [37].

2.4. Genomic and cellular analyses

Based on the data presented by Thorsson et al. [36] patients were divided into *EGOT*-low (negative z-score) and *EGOT*-high (positive z-score) and association of *EGOT* with cellular features including TCGA subtype, proliferation ratio, wound healing ability, stromal fraction, intratumor heterogeneity were checked.

2.6. Statistical analyses

We used GraphPad Prism 8 (GraphPad, San Diego, CA, USA) and verified using Statistica 12.0 (StatSoft) for statistical analysis. In all analyses Shapiro–Wilk normality test was used for checking the value distribution. Next, depending on the distribution t-test or Mann–Whitney U test were used for comparison of two-group analyses. For the analysis of three or more groups one way ANOVA with proper post-test was calculated. In all analyses, $p < 0.05$ was used to determine statistical significance [6,37].

3. Results

3.1. EGOT is a potential prognostic marker for breast, head and neck, kidney and liver carcinomas

The association between *EGOT* expression levels and patients' survival was checked (Tab.2) Better overall survival for patients with high expressions of *EGOT* was observed in the case of breast invasive carcinoma (131 vs 115 days, HR = 0.63, p = 0.0042), head and neck squamous cell carcinoma (65 vs 35 days, HR = 0.69, p = 0.0062), kidney renal clear cell carcinoma (118 vs 74 days, HR = 0.63, p = 0.017), kidney renal papillary cell carcinoma (>130 vs 98 days, HR = 0.52, p = 0.037) and cutaneous melanoma (106 vs 62 days, HR = 0.76, p = 0.039) compared to the group of patients with low expressions of this lncRNA. However, in the case of liver hepatocellular carcinoma (48 vs 70 days, HR = 1.48, p = 0.027) and

mesothelioma (14 vs 22 days, HR = 1.61, p = 0.044), patients with lower expressions of *EGOT* have longer survival than patients with higher expressions (Fig. 2 and Tab.1.)

Based on available databases, ENCORI Pan-Cancer Analysis Platform and the UALCAN database, we analyzed and compared expression data of *EGOT* lncRNA in 17 different cancers (Tab. 1). In the case of eleven tumors, we indicated significant changes (p > 0.05) between normal and cancer samples (Tab. 1). For BRCA (1.45 vs 1.41, p = 0.00042), HNSC (0.19 vs 0.26, p = 0.00011), KIRC (2.66 vs 4.43, p = 1.1e-5), PRAD (0.24 vs 0.49, p = 2.9e-9) and KICH (0.82 vs 6.28, p = 7.8e-18) expressions of *EGOT* were down-regulated. For six types of cancer: CHOL (0.53 vs 0.08, p = 0.0012), COAD (0.17 vs 0.03, p = 2.5e-7), ESCA (0.2 vs 0.06, p = 0.0054), LUAD (0.59 vs 0.09, p = 1.8e-7), STAD (0.13 vs 0.08, p = 0.031) and THCA (0.49 vs 0.19, p = 8.4e-7) *EGOT* expression was up-regulated in cancer samples compared to the normal tissue samples. All data is presented in Fig. 2 and Table 2. Moreover, we checked the median expression of *EGOT* in tumor and normal samples, based on GEPIA2 database for BRCA, HNSC, KIRC, and LIHC, and indicated that higher fold change (FC) in expression levels of *EGOT* is characteristic for LIHC (FC = 1.3333) and KIRC (FC = 1.0185), and lower for BRCA (FC = 0.6795) and HNSC (FC = 0.4706), Figure 2C.

3.2. EGOT is associated with cellular pathways and processes important with cancer development and progression

Next, we analyzed the list of Spearman's correlation (R < -0.1 and R > 0.1) for genes' list with *EGOT* taken from cBioPortal. Cellular pathways and processes were assessed based on the negatively and positively correlated genes with *EGOT* for four types of cancers. From the analysis of REACTOME results we identified common for BRCA, HNSC, KIRC, and LIHC, and specific for type of cancer changes in processes. In the case of positively correlated genes with *EGOT*, patients displayed changes in: GTPases cycle, tissue architecture (including changes in collagen), cellular interactions and structures, O-glycosylation and N-glycan synthesis, signaling pathways and processes connected with genetic defects, as well as changes in mRNA regulation and RNA polymerase activity. The negatively correlated genes were associated with ubiquitination and protein degradation, metabolism (specifically ornithine decarboxylase (ODC) and amino acids), regulation of cell cycle, signal transduction, interaction with cytoskeleton, GTPase activity, RNA polymerase dynamics dynamics, cross-presentation of antigens and DNA repair mechanisms. All results are presented in Figure 3A and 3B.

3.3. Expression level of EGOT depends on the type of cancer and its immune subtype and cellular features for specified cancers

Next, we determined the characteristics of *EGOT* levels in BRCA, HNSC, KIRC, and LIHC, depending on the immune subtypes based on the TCGA classification. We observed no differences in expression levels in the case of the head and neck and liver carcinomas ($p > 0.05$). Only in the case of BRCA the most changes in the expression levels of *EGOT* were indicated between immune subtypes: C1 vs. C3 (-0.1051 vs. 0.6433; $p < 0.0001$, C2 vs. C3 (-0.3752 vs. 0.6433; $p < 0.0001$) and C3 vs. C4 (0.6433 vs. -0.1159; $p < 0.0001$). Moreover, in kidney renal clear cell carcinoma expression levels of *EGOT* significantly differ between: C1 vs. C3 (-1.762 vs. 0.2717; $p < 0.01$) and C2 vs. C3 (-0.5846 vs. 0.2717; $p < 0.0059$) immune subtypes, Figure 4A.

Next, we checked the expression level of *EGOT* lncRNA depending on the TCGA cancer subtypes. Differences were observed for all four types of analyzed cancers. In the case of BRCA, Basal vs. Luminal A, Basal vs. Luminal B, Basal vs. Normal, HER2 vs. Luminal A, Luminal A vs. Luminal B ($p < 0.0001$), Luminal A vs. Normal ($p = 0.0002$) and HER2 vs. Normal ($p = 0.0021$) with median expressions -0.9107, 0.3955, -0.3494, 0.0261 and -0.6712, respectively. For HNSC we indicated changes between subtypes: Atypical vs. Basal and Atypical vs. Classical (with median 0.5189, -0.7936, -0.7529; $p < 0.0001$), Atypical vs. Mesenchymal (0.5189 vs. -0.2342; $p = 0.0006$), and Basal vs. Mesenchymal (-0.7936 vs. -0.2342; $p = 0.0084$). Similarly, for four TCGA subtypes, changes in *EGOT* expression were indicated for KIRC, and this included KIRC vs. KIRC3 ($p = 0.0334$), KIRC1 vs. KIRC2 ($p = 0.0033$), KIRC1 vs. KIRC3 ($p < 0.0001$) and KIRC1 vs. KIRC4 ($p = 0.0015$) with median expression 0.3339, -0.4254, 0.4887, 0.2342 and 0.127, respectively. Whereas for LIHC, changes in the expression levels of *EGOT* were observed only between Cluster2 vs. Cluster3 (-0.8772 vs. -0.0198; $p = 0.0199$) and LIHC vs. Cluster2 (0.5972 vs. -0.8772; $p = 0.0151$). Moreover, cellular features such as proliferation, wound healing ability, stromal fraction and intratumor heterogeneity were checked. The lower *EGOT* expression levels were associated with higher proliferation (0.4452 vs. -0.1037; $p < 0.0001$, and -1.021 vs. -1.176; $p = 0.0058$), and wound healing ability (0.08050 vs. -0.03950; $p < 0.0001$, and -0.1970 vs. -0.2615; $p < 0.0001$) than groups of BRCA and KIRC patients' with higher expression of this lncRNA. In the case of HNSC, higher *EGOT* expression was associated with higher proliferation (0.6896 vs. 0.6104; $p = 0.0058$), and no differences in wound healing ability were noticed ($p > 0.05$). The third parameter, stromal cell fraction within the analyzed sample, was higher in the case

of KIRC patients with lower expression levels of *EGOT* (0.5300 vs. 0.4100; $p < 0.0001$), and no significant differences were observed for the rest of the types of analyzed cancers ($p > 0.05$). Moreover, we indicated lower intratumor heterogeneity was associated with higher *EGOT* expression only in the case of BRCA and KIRC (0.06000 vs. 0.1300; $p < 0.0001$, and 0.01000 vs. 0.04000; $p = 0.0012$). Surprisingly, for LIHC no differences ($p > 0.05$) for all analyzed parameters were noticed, Figure 4C.

4. Discussion

In our opinion, despite the improvement of treatment protocols in oncology and progress in technology, e.g., in radiotherapy and cancer diagnostic methods, we have still not observed a spectacular breakthrough in personalized oncology [39-46]. However, the introduction of RNA-based biomarkers, especially those RNA molecules that take part in epigenetics mechanisms, could be a big step towards personalized oncology [1-5]. However, before it happens, many issues need to be solved, including creation and validation of the diagnostics panels of specific RNA molecules, which give us information about patients [7,9,33,34].

Our study is the first comprehensive description of the biological and clinical role of *EGOT*. Numerous recent studies have been dedicated to understanding the role of *EGOT* in glioma, breast cancer, gastric cancer, head and neck and other malignancies [12-25] and in viral infections, including HCV [29,30]. Moreover, our observation is supported by Tomasr S. results (PhD dissertation, 2013) where, based on microarray analysis of 65 samples of oropharyngeal HPV-positive and HPV-negative cancer, changes of *EGOT* expression levels depending on HPV status and viral activity were observed [38]. In the case of HNSC, expression of *EGOT* is slightly down-regulated in the whole group of patients (HPV positive and HPV negative), depending on tumor grade and location. EGOT is only up-regulated in grade 4 tumors and those located in the pharynx (including nasopharynx, oropharynx and hypopharynx). *EGOT* expression levels were found to vary according to age, N-stage, grade, lymph node dissection and HPV infection. Patients with higher levels of *EGOT* expression have longer survival rates, including both disease-free survival and overall survival outcomes. Further analysis revealed that *EGOT*s' targets are associated with cell division, proliferation, protein modification, drug response and cell motility [6]. However, further studies are needed to elucidate the function of EGOT in HNSC as well as other types of cancers.

Pathway analysis based on gene correlation with *EGOT* revealed that genes correlated with this lncRNA involve cell division, proliferation, protein modification, drug response and cell motility. Based on our findings we suggest that the *EGOT* is involved in the progression of HSNCC and seems particularly associated with virus-related forms of HNSCC [6,47]. However, validation of our results using different data and a larger group of patients is needed. Similarly, Xu et al. found that *EGOT* expression was lower in breast cancer cells compared to non-cancerous samples and varies according to the molecular subtypes of breast cancer. Furthermore, it was also indicated that tumor size, lymph node metastasis and Ki-67 expression were positively correlated with low *EGOT* expression. This evidence shows that the down-regulation of *EGOT* is involved in the progression of more invasive types of cancer [14]. In glioma, the expression of *EGOT* is significantly lower in the cancer than in the adjacent non-cancerous tissues [19]. An *in vitro* study determined that the over-expression of *EGOT* inhibits cell proliferation and migration and promotes cell apoptosis by increasing protein expression levels of caspase-3, caspase-9 and cytochrome c in U251 and U87 glioma cell lines [19]. Similarly, in renal cell carcinoma, the expression of *EGOT* is down-regulated in tumor samples compared to paired, healthy tissues. *In vitro* study found that the up-regulation of *EGOT* expression suppresses proliferation, migration and invasion and induces apoptosis in 786-O and ACHN renal cell lines [18]. Taken together, these results suggest that *EGOT* serves as a suppressor gene. Surprisingly, our analysis of clinical pathology parameters in patients with HNSC indicated that a low expression level of *EGOT* is observed in the group of patients with lower N-stage, lower grade, and higher age of patients. It should be emphasized that some authors indicated that *EGOT* could be oncogene lncRNA. Based on the study performed by Peng et al. using patients' gastric carcinoma samples and cell line model showed that *EGOT* is up-regulated and high expression levels were correlated with more aggressive forms of cancers. Moreover, it was indicated that changes in *EGOT* levels influence the Hedgehog pathway, but the mechanism of this phenomenon was not shown [17]. Furthermore, down-regulated *EGOT* expression *in vitro* results in the inhibition of the hedgehog signaling pathway, cell proliferation and cycle progression arrest in the case of gastric cancer and breast cell lines [13,17]. Based on the TCGA data of HNSC, it was observed that patients with high *EGOT* expression levels have better prognosis (longer DFS and OS) than those with low expression [6]. Similarly, in the case of breast cancer, patients with lower expression levels of EGOT displayed shortened OS time [14]. However, the opposite pattern was observed for gastric cancer, in which higher *EGOT* expression was associated with shorter survival times [17]. The genes that were up-regulated in the high expression group are involved in the regulation of cellular processes, including

differentiation, adhesion, developmental process, cell communication, signal transduction, division and proliferation, protein phosphorylation and other modifications, cellular component organization, cellular homeostasis, drug response and cell motility. It was observed in the group of down-regulated genes that grouped in processes connected with cell cytoskeleton and filaments, localization/binding, cellular transport and protein activity.

The changes in these processes indirectly or directly influence the treatment response and survival of patients with HNSC. It must also be noted that the location of HNSC is a crucial clinical factor in terms of treatment strategy and survival prediction. The observed up-regulation of *EGOT* expression in the pharyngeal cancers is probably due to HPV infection, which is characteristic of the oropharynx, such as the tonsils and base of the tongue, but is sometimes also associated with other locations [6]. Indeed, our observations confirmed that *EGOT* expression is mainly up-regulated in HPV p16-positive HNSC. It has been shown that the expression of some other lncRNAs is associated with viral infections [29-31]. Another study has shown that *EGOT* expression is up-regulated in HPV-positive HNSC [39]. However, the authors did not propose a mechanism of *EGOT* function in HPV infection, so the role of this lncRNA in viral infection remains unclear [39]. Based on the patient's samples and cell lines, Carnero et al. indicated the lncRNA *EGOT* is involved and required for hepatitis C virus replication. They also observed that cells with lower *EGOT* levels have lower content of viral genomes [30]. It is proposed that the viral replication process in the host cell cytoplasm causes up-regulation of *EGOT*, which is required to overcome the cellular mechanism of antiviral response [30]. It was observed that EGOT is up-regulated after exposure to dsRNA or synthetic analogs and viral RNA as well as after exposure of TNFα (tumor necrosis factor α). In the proposed mechanism, expression of *EGOT* is stimulated by NF-kB (nuclear factor-κB), in the response to TNFα, which binds to the promoter of gene coding lncRNA *EGOT* [30]. These results suggested that *EGOT* is involved in the progression of HNSC [6]. Furthermore, it seems likely that the role of *EGOT* is connected to HPV infection, given the association between high *EGOT* expression levels and pharyngeal tumors, younger patients, better DFS and OS and p16 expression, as these are all characteristic of HPV-positive HNSC cases [6]. We supposed that *EGOT* could potentially be a new biomarker of HPV infection and probably has an important role in viral response and biology of HPV-positive oropharynx cancers. It is known that *EGOT* plays some regulatory functions as the so-called "molecular sponge" and by binding miRNAs, it causes a change in their level in the cell, which ultimately affects the expression level of other transcripts, including mRNA [20-22].

However, it is not defined for all of the types of cancers presented in this study, and we see the gap in mechanism between *EGOT* and observed biological and clinical features associated with this lncRNA.

We believe that defining the role of *EGOT* will allow us to better understand and characterize BRCA, HNSC, KIRC and LIHC, and to answer the question of whether *EGOT* lncRNA will be able to become a biomarker of detection, treatment response, or disease stage in the future. However, the exact connection of *EGOT* in the case of BRCA, HNSC, KIRC, and LIHC has not been clearly understood so far. One of the possibilities that connected HNSC and LIHC cancers and changes in *EGOT* expression is viral activity and immunological response to viral infection. The role of HPV, mostly HPV-16 and HPV-18, in HNSC, and the hepatitis B virus (HBV), the hepatitis C virus (HCV), and the hepatitis D virus (HDV) in the case of LIHC development is well known [48-49]. Recent studies about KIRC postulated the possible role of endogenous retrovirus (ERV) reactivation in renal cell carcinoma oncogenesis which is connected with hypotheses about it immunogenicity and, what is more fascinating, with the response to the treatment based on inhibitors of immune checkpoints [50]. In the case of BRCA, the role of some viruses in BRCA-oncogenesis remains inconclusive and enigmatic. However, Epstein-Barr virus (EBV), as well as bovine leukemia virus (BLV), are indicated as potentially causing this type of cancer, but authors underline that it is not so common and viral infection could be one of the factors in the oncogenesis process [51]. We observed that the results of the association of *EGOT* with BRCA are also connected with virus-mediated changes in the cell genome or epigenome as well as changes in the tumor microenvironment, including the immune response. However, strong evidence is needed for this statement.

Our study is the first comprehensive description of lncRNA *EGOT* across 17 different cancer types included in the TCGA project. Based on our results we conclude that:

1. Expression levels of lncRNA *EGOT* are higher in non-cancerous samples in the case of HNSC and lower in the case of KIRC and LIHC in comparison to cancer samples;

2. Higher expression levels of *EGOT* is associated with better overall survival for patients with lower levels of this lncRNA for BRCA, HNSC and KIRC cancers compared to those with lower levels of this lncRNA;

3. For all analyzed cancer types, patients exhibited changes in genes positively correlated with lncRNA *EGOT* which were associated with GTPases in signaling pathways, alterations in extracellular collagen, changes in glycolytic pathways and some genetic defects important for cancerogenesis. In contrast, negatively correlated genes with lncRNA *EGOT*

were linked to changes in cell cycle regulation, protein homeostasis, metabolic processes and DNA repair mechanisms.

However, we acknowledge some limitations of our study, despite utilizing various analytical platforms with the TCGA data concerning lncRNA *EGOT*. It should be noted that some of the presented results were calculated by us. Nevertheless, all results are based on the same project and data, which has positive and negative impact. The advantage is that generated data were obtained under the same condition and quality control. Moreover, the TCGA data is the biggest collection of cancer samples available. Unfortunately, a limitation of our study is the lack of other datasets similar to the TCGA for validation purposes.

In summary, our results provide an excellent starting point for further studies by other teams that can validate these findings based on their own data or *in vitro* cell models.

Authors' Contributions: Authors' individual contributions:
conceptualization - TK
methodology - TK
investigation - TK, KG, JK-M, PP, AF, UK, AM-D, DP-H, MN, MJ-P, JK, AP, AZ, EW
data curation - TK, KG, JK-M
writing - original draft preparation - TK, KG, JK-M, PP, ZC, AF, UK, AM-D, DP-H, MN, MJ-P, JK, AZ, EW
writing - review and editing - TK, KG, JK-M, PP, ZC, AF, UK, AM-D, DP-H, MN, MJ-P, JK, AP, AZ, EW
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Conflicts of Interest: The authors declare that there is no conflict of interest regarding the publication of this paper. All authors read and approved the final manuscript.

Availability of data and materials: The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request. Raw data are available on the Encori, Ualcan and cBioPortal databases.

Ethics approval: Study is based on analysis of freely available data sets and does not need any ethics committee's agreement, and does not violate the rights of other persons or institutions.

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Figure and Table description section:

Figure 1. Patient characteristics used in the study among breast invasive carcinoma (BRCA), head and neck squamous cell carcinoma (HNSC), kidney renal clear cell carcinoma (KIRC) and liver hepatocellular carcinoma (LIHC) type of cancers. Graphs taken from cBioPortal; NA - not analyzed.

Figure 2. Characteristics of *EGOT* across the different types of cancers analyzed during the TCGA project. Association of *EGOT* expression levels with patients' overall survival (OS) taken from Starbase 3.0 database (A), and OS and relapse-free survival (RFS) taken from GEPIA2 database (B); log rank test, low and high groups divided based on the median

expression; green and red solid lines show survival, and dashed lines indicate 95% CI, HR - hazard ratio; C) Median expression of *EGOT* in tumor and normal samples, graph taken from GEPIA2 database; and D) differences in expression level of *EGOT* between cancer and normal samples in different types of tumors; graphs from ENCORI database, modified; the graphs represent the median of the value presented as fragments per kilobase million+0.01 (FPKM+0.01); BRCA - breast invasive carcinoma, HNSC - head and neck squamous cell carcinoma, KIRC - kidney renal clear cell carcinoma, LIHC - liver hepatocellular carcinoma; n - number of cases, ns - not significant, **** $p < 0.0001$, * $p < 0.05$ considered as significant

Figure 3. Assessment of cellular pathways and processes based on the negatively and positively correlated genes with *EGOT* for breast invasive carcinoma (BRCA), head and neck squamous cell carcinoma (HNSC), kidney renal clear cell carcinoma (KIRC) and liver hepatocellular carcinoma (LIHC). Spearman's correlation ($R < -0.1$ and $R > 0.1$) of genes' list with *EGOT* taken from cBioPortal and analyzed using the REACTOME tool with p-value ≤ 0.05 as cut off value

Figure 4. Characteristics of *EGOT* in breast invasive carcinoma (BRCA), head and neck squamous cell carcinoma (HNSC), kidney renal clear cell carcinoma (KIRC) and liver hepatocellular carcinoma (LIHC), depending on (A) immune and (B) the TCGA subtypes, (C) cellular features such as proliferation, wound healing ability, stromal fraction and intratumor heterogeneity, ns - not significant, **** $p < 0.0001$, *** $p < 0.001$, ** $p < 0.01$ and * $p < 0.05$ considered as significant

Table 1. Expression levels of *EGOT* in cancer and normal samples in different types of tumors; FDR - false discovery rate; $p < 0.05$ considered as significant

Table 2. Association of *EGOT* expression level and patients' survival; HR - hazard ratio; $p < 0.05$ considered as significant

lncRNA *EGOT*

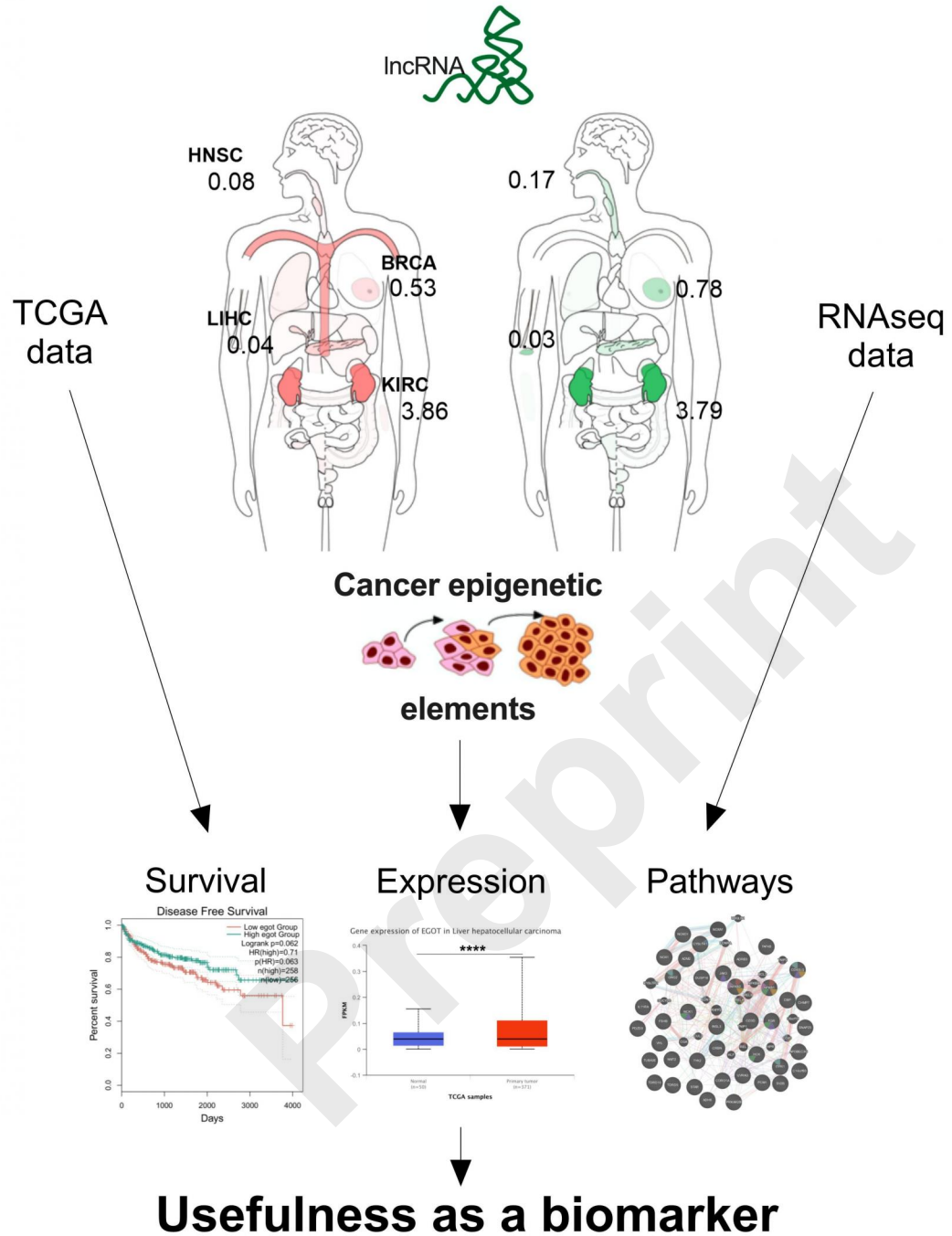


Table 1. Expression levels of *EGOT* in cancer and normal samples in different types of tumors; FDR - false discovery rate; $p < 0.05$ considered as significant

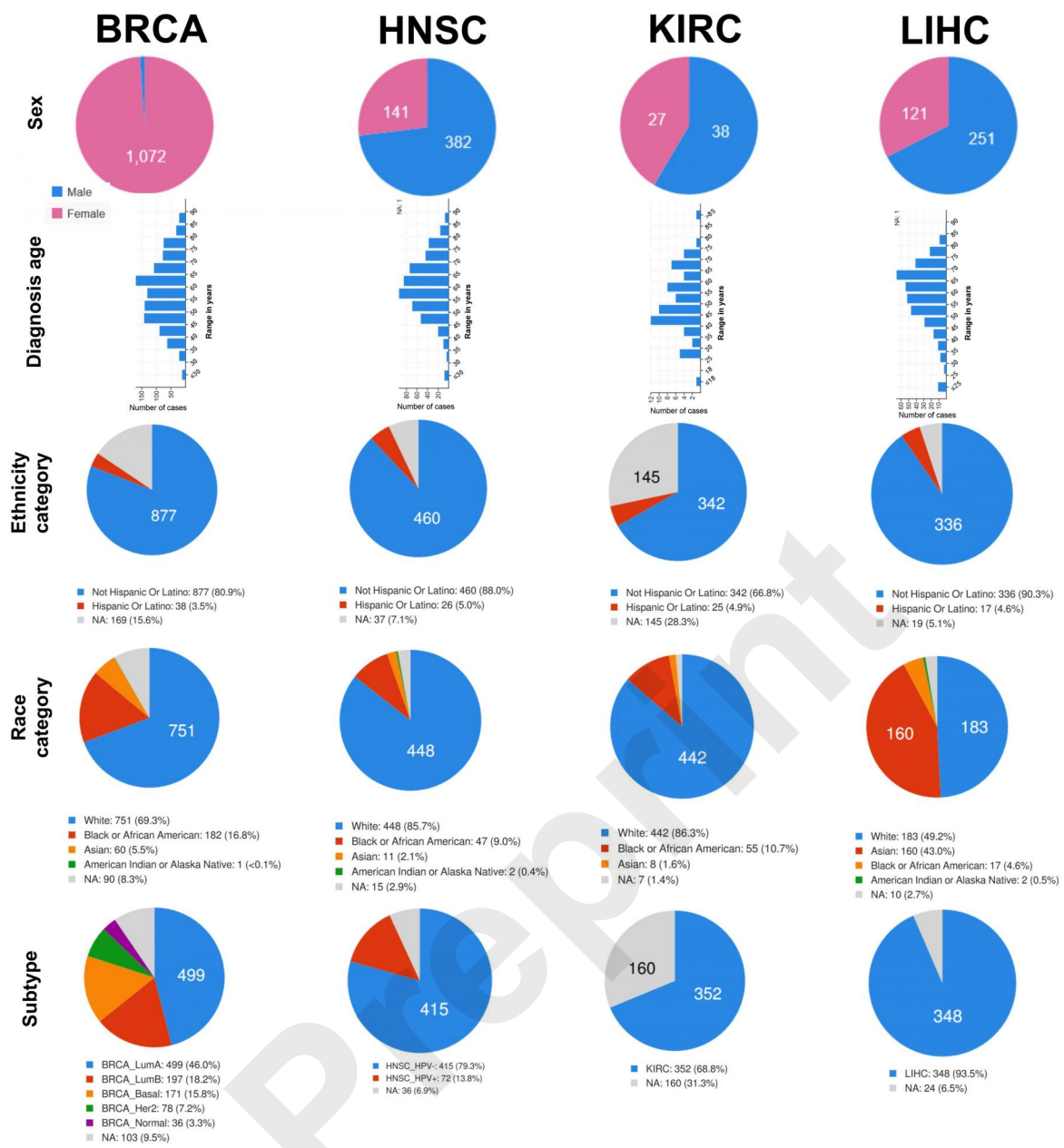
Cancer		**Cancer cases**	**Normal cases**	**Cancer expression**	**Normal expression**	**Fold change**	**P-value**	**FDR**
Kidney Chromophobe	KICH	65	24	0.82	6.28	0.13	**7.8e-18**	2.3e-16
Prostate Adenocarcinoma	PRAD	499	52	0.24	0.49	0.5	**2.9e-9**	3.2e-8
Lung Adenocarcinoma	LUAD	526	59	0.59	0.09	6.29	**1.8e-7**	9.4e-7
Colon Adenocarcinoma	COAD	471	41	0.17	0.03	5.97	**2.5e-7**	1.6e-6
Thyroid Carcinoma	THCA	510	58	0.49	0.19	2.63	**8.4e-7**	4.2e-6
Kidney Renal Papillary Cell Carcinoma	KIRP	289	32	2.66	4.43	0.6	**1.1e-5**	6.7e-5
Head and Neck Squamous Cell Carcinoma	HNSC	502	44	0.19	0.26	0.75	**0.00011**	0.0005
Breast Invasive Carcinoma	BRCA	1104	113	1.45	1.41	01.03	**0.00042**	0.0011
Cholangiocarcinoma	CHOL	36	9	0.53	0.08	6.41	**0.0012**	0.0043
Esophageal Carcinoma	ESCA	162	11	0.2	0.06	3.15	**0.0054**	0.036
Stomach Adenocarcinoma	STAD	375	32	0.13	0.08	1.74	**0.031**	0.063
Bladder Urothelial Carcinoma	BLCA	411	19	0.18	0.16	01.09	0.06	0.18
Kidney Renal Clear Cell Carcinoma	KIRC	535	72	4.98	4.39	1.13	0.1	0.15
Pancreatic Adenocarcinoma	PAAD	178	4	0.75	1.47	0.51	0.15	0.66
Lung Squamous Cell Carcinoma	LUSC	501	49	0.36	0.13	2.76	0.16	0.26
Liver Hepatocellular Carcinoma	LIHC	374	50	0.18	0.07	2.41	0.17	0.29

Uterine Corpus Endometrial Carcinoma	UCEC	548	35	0.27	0.22	1.22	0.88	0.92

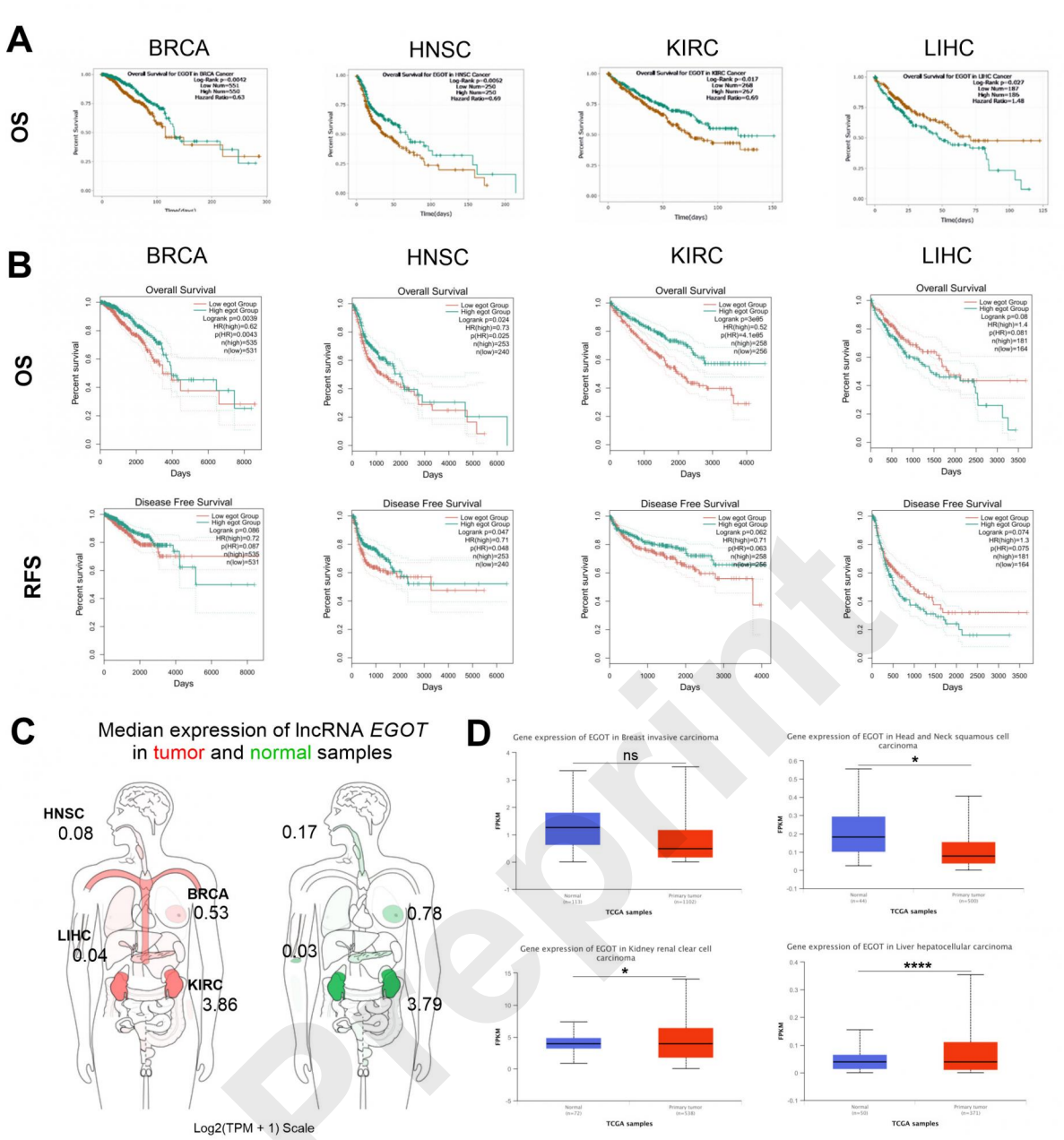
Table 2. Association of *EGOT* expression level and patients' survival; HR - hazard ratio; $p < 0.05$ considered as significant

Cancer type		**Cases**	**Median**	**Coefficient**	**HR**	**P-value**
Breast Invasive Carcinoma	BRCA	1104	0.57	-0.46	0.63	**0.0042**
Head and Neck Squamous Cell Carcinoma	HNSC	502	0.09	-0.37	0.69	**0.0062**
Kidney Renal Clear Cell Carcinoma	KIRC	535	4.13	-0.36	0.69	**0.017**
Liver Hepatocellular Carcinoma	LIHC	374	0.05	0.39	1.48	**0.027**
Kidney Renal Papillary Cell Carcinoma	KIRP	289	2.15	-0.65	0.52	**0.037**
Skin Cutaneous Melanoma	SKCM	471	0.01	-0.28	0.76	**0.039**
Mesothelioma	MESO	86	0.17	0.48	1.61	**0.044**
Uterine Corpus Endometrial Carcinoma	UCEC	548	0.12	0.27	1.31	0.19
Pancreatic Adenocarcinoma	PAAD	178	0.53	0.27	1.31	0.2
Testicular Germ Cell Tumors	TGCT	156	0.02	1.42	4.15	0.2
Adrenocortical Carcinoma	ACC	79	0.01	-0.47	0.63	0.23
Thymoma	THYM	119	0.06	0.72	02.06	0.29
Cervical Squamous Cell Carcinoma and Endocervical Adenocarcinoma	CESC	306	0.2	-0.24	0.78	0.31
Bladder Urothelial Carcinoma	BLCA	411	0.04	0.15	1.16	0.32
Ovarian Serous Cystadenocarcinoma	OV	379	0.22	0.12	1.13	0.35

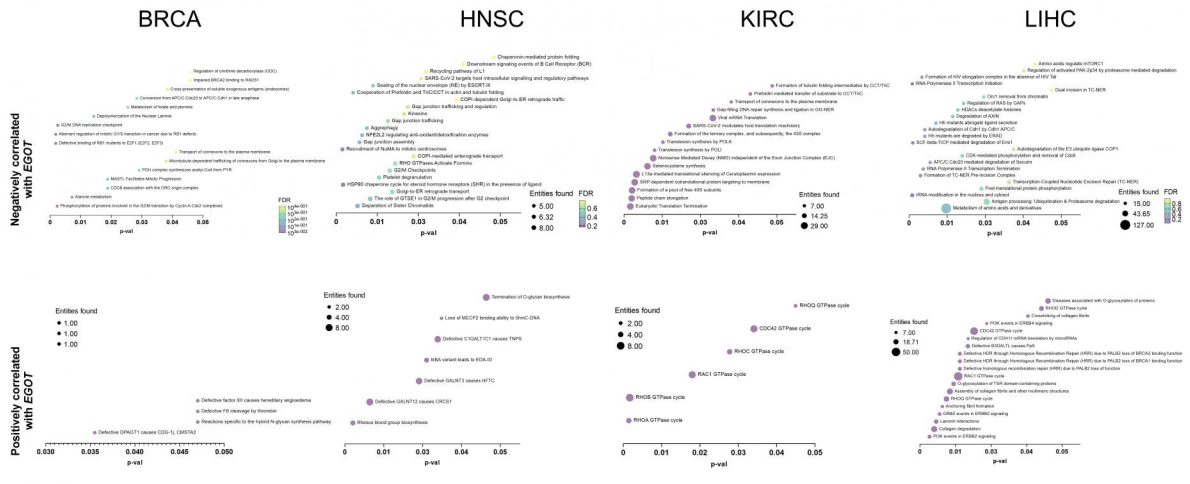
Cholangiocarcinoma	CHOL	36	0.35	-0.37	0.69	0.44
Stomach Adenocarcinoma	STAD	375	0.08	0.13	1.14	0.44
Thyroid Carcinoma	THCA	510	0.34	-0.35	0.7	0.48
Acute Myeloid Leukemia	LAML	151	2.1	0.14	1.16	0.49
Pheochromocytoma and Paraganglioma	PCPG	183	0.01	-0.48	0.62	0.51
Rectum Adenocarcinoma	READ	167	0.06	-0.25	0.78	0.52
Colon Adenocarcinoma	COAD	471	0.06	0.08	01.09	0.67
Sarcoma	SARC	263	0.04	-0.08	0.92	0.68
Uterine Carcinosarcoma	UCS	56	0.05	-0.1	0.9	0.76
Lung Adenocarcinoma	LUAD	526	0.2	-0.04	0.96	0.77
Lung Squamous Cell Carcinoma	LUSC	501	0.12	-0.03	0.97	0.83
Esophageal Carcinoma	ESCA	162	0.11	0.05	01.05	0.83
Kidney Chromophobe	KICH	65	0.26	-0.14	0.87	0.84
Prostate Adenocarcinoma	PRAD	499	0.11	0.09	1.1	0.88
Lymphoid Neoplasm Diffuse Large B-cell Lymphoma	DLBC	48	0.02	0.02	01.02	0.98
Brain Lower Grade Glioma	LGG	529	0	0	0	1
Uveal Melanoma	UVM	80	0	0	0	1



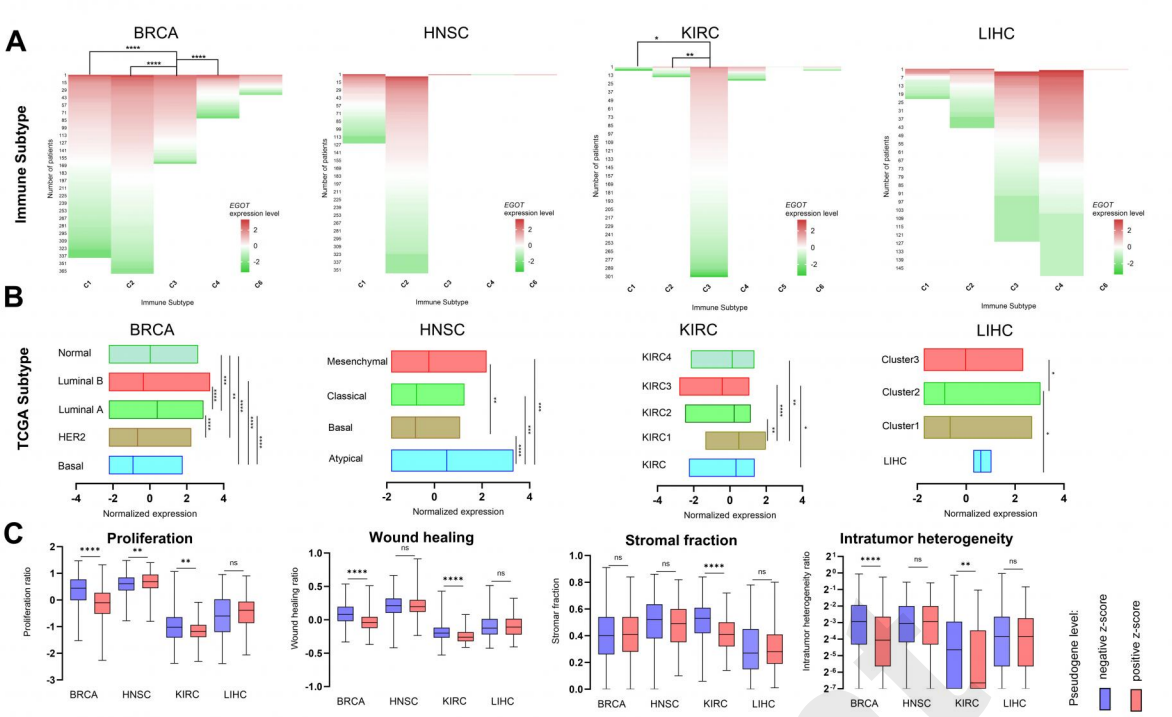
Patient characteristics used in the study among breast invasive carcinoma (BRCA), head and neck squamous cell carcinoma (HNSC), kidney renal clear cell carcinoma (KIRC) and liver hepatocellular carcinoma (LIHC) type of cancers. Graphs taken from cBioPortal; NA - not analyzed.



Characteristics of EGOT across the different types of cancers analyzed during the TCGA project. Association of EGOT expression levels with patients' overall survival (OS) taken from Starbase 3.0 database (A), and OS and relapse-free survival (RFS) taken from GEPIA2 database (B); log rank test, low and high groups divided based on the median expression; green and red solid lines show survival, and dashed lines indicate 95% CI, HR - hazard ratio; C) Median expression of EGOT in tumor and normal samples, graph taken from GEPIA2 database; and D) differences in expression level of EGOT between cancer and normal samples in different types of tumors; graphs from ENCORI database, modified; the graphs represent the median of the value presented as fragments per kilobase million+0.01 (FPKM+0.01); BRCA - breast invasive carcinoma, HNSC - head and neck squamous cell carcinoma, KIRC - kidney renal clear cell carcinoma, LIHC - liver hepatocellular carcinoma; n - number of cases, ns - not significant, **** $p < 0.0001$, * $p < 0.05$ considered as significant



Assessment of cellular pathways and processes based on the negatively and positively correlated genes with EGOT for breast invasive carcinoma (BRCA), head and neck squamous cell carcinoma (HNSC), kidney renal clear cell carcinoma (KIRC) and liver hepatocellular carcinoma (LIHC). Spearman's correlation (R < -0.1 and R > 0.1) of genes' list with EGOT taken from cBioPortal and analyzed using the REACTOME tool with p-value ≤ 0.05 as cut off value



Characteristics of EGOT in breast invasive carcinoma (BRCA), head and neck squamous cell carcinoma (HNSC), kidney renal clear cell carcinoma (KIRC) and liver hepatocellular carcinoma (LIHC), depending on (A) immune and (B) the TCGA subtypes, (C) cellular features such as proliferation, wound healing ability, stromal fraction and intratumor heterogeneity, ns - not significant, **** $p < 0.0001$, *** $p < 0.001$, ** $p < 0.01$ and * $p < 0.05$ considered as significant