Clustering Solid Tumors Based on Autophagy-Related Genes

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There has been a boost in autophagy reports due to its role in cancer progression and its association with tumor resistance to treatment. The availability of large cancer datasets has provided an extensive evidence-based approach to understanding the role of autophagy-related genes in various human cancers and their clinical implications, including cancer progression, development, and treatment response.

autophagy cancer gene Data analysis

1. Introduction

To assess the clustering of solid tumors, researchers utilized the expression levels of autophagy-related genes. To ensure robust observations, researchers focused on solid tumor types with more than 100 tumor and control samples. Employing this approach, researchers generated a UMAP plot that revealed three distinct clusters among the sixteen solid tumors analyzed (**Figure 1**A). The identified clusters were as follows: Cluster 0 comprised BRCA, KIRC, KIRP, LGG, KIHC, LUAD, LUSC, and PRAD; Cluster 1 included COAD, ESCA, PAAD, and STAD; and Cluster 2 consisted of GBM, OV, SKCM, and TGCT. Notably, cluster 0 grouped tissues with similar genetic or anatomical profiles, such as BRCA-PRAD, KIRC-KIRP, or LUAD-LUSC. Cluster 1 predominantly encompassed gastrointestinal tumors, while Cluster 2 included TGCT and OV, which are tumors from reproductive organs. To identify differentially expressed genes characterizing these clusters, researchers identified 18 genes that primarily distinguished Clusters 0 and 1. Cluster 2 exhibited decreased levels of these markers (**Figure 1**B), and therefore it will not be analyzed in further detail herein. Nevertheless, it is important to note that autophagy profiles have been induced and studied on SKCM, GBM, and OV models with anti-tumoral effects [1][2][3][4]. In addition, for GBM and SKCM, there is possible to suggest that expression similarities in non-pivotal genes could be originated at their division from the ectoderm, as was demonstrated for the *P2X7* receptor [5]. Then, these findings could support the evolutive hypothesis of cancer as an embryological phenomenon [6].

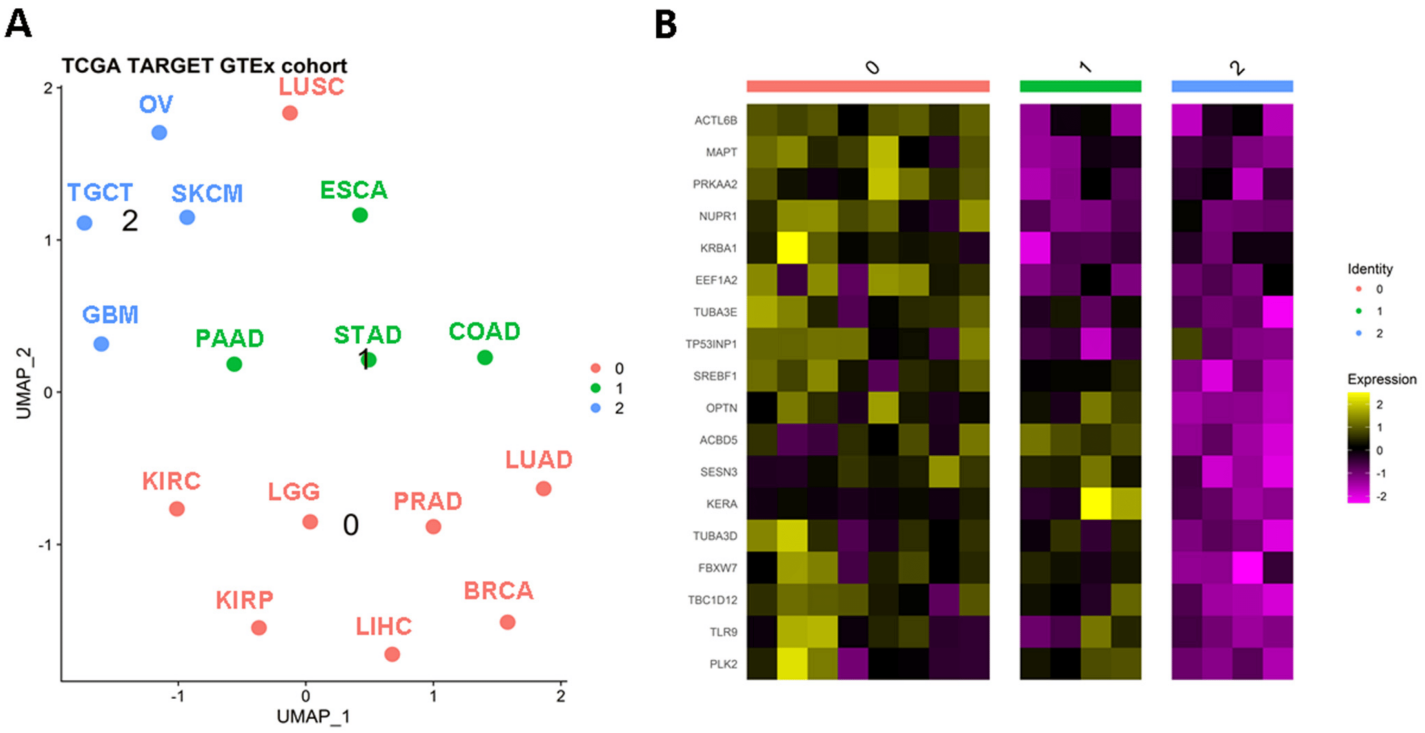

Figure 1. Autophagy-related genes can stratify solid tumors. (**A**) Clusterization of solid tumors based on the differential expression of autophagy genes. After a UMAP analysis, it is possible to recognize three classifications (**B**) of relevant tumors based on the expression of autophagy genes. BRCA: breast invasive carcinoma; COAD: colon adenocarcinoma; ESCA: esophageal carcinoma; GBM: glioblastoma multiforme; KIRC: kidney renal clear cell carcinoma; KIRP: kidney renal papillary cell carcinoma; LGG: brain lower grade glioma; LIHC: liver hepatocellular carcinoma; LUAD: lung adenocarcinoma; LUSC: lung squamous cell carcinoma; OV: ovarian serous cystadenocarcinoma; PAAD: pancreatic adenocarcinoma; PRAD: prostate adenocarcinoma; SKCM: skin cutaneous melanoma; STAD: stomach adenocarcinoma; TGCT: testicular germ cell tumors.

2. Autophagy Regulators Specific to Cluster 0

Researchers' analysis revealed that the genes *ACTL6B*, *MAPT*, *PRKAA2*, *NUPR1*, *KRBA1*, *EEF1A2*, *TUBA3E*, and *TP53INP1* specifically characterized cluster “0” through their overexpression. Furthermore, researchers observed that these genes exhibited upregulated levels in tumors belonging to Cluster “0” compared to their normal adjacent tissues. While the limited number of normal-adjacent samples in the TCGA data introduces potential biases and limitations, the majority of the putative markers for Cluster “0” could be validated using the UALCAN tool [7].

2.1. Protein and Mutational Features of Relevant Genes for Cluster 0

To gain insight into the protein products of these genes, researchers utilized the UALCAN tool to examine their change in proteins with data from the Clinical Proteomic Tumor Analysis Consortium (CPTAC) cohort. In the LIHC dataset, researchers found a comparable group of normal-adjacent samples, which demonstrated the upregulation

of EEF1A2, NUPR1, and MAPT proteins in the tumor group. However, there were some inconsistencies. For instance, while the *PRKAA2* gene exhibited notable overexpression in LIHC, its corresponding protein was significantly downregulated. This disparity suggests the importance of considering the mutational profile of these genes or post-translational events on the produced proteins. Based on data from cBioPortal [8] of the TCGA datasets belonging to Cluster "0", these genes exhibited a low frequency of mutations (1.1–5%), implying that somatic mutations may not play a significant role in dysregulating the relationship between autophagy-related genes and their corresponding proteins.

2.2. Previous Research on Relevant Genes for Cluster 0

According to the MSigDB, the *NUPR1* and *PRKAA2* genes participate in macroautophagy and its regulation, while *MAPT* serves as an autophagy regulator, *TP53INP1* contributes to autophagosome organization, and *ACTL6B* and *KRBA1* are involved in the autophagy-related network. Additionally, other genes are associated with less-studied forms of autophagy. For example, *EEF1A2* is linked to chaperone-mediated autophagy and its regulation, *PRKAA2* is involved in lipophagy-related pathways, and *TUBA3E* is associated with aggrephagy.

Previous studies have highlighted the significant role of *NUPR1* in macroautophagy and its impact on the aggressiveness and treatment resistance of specific tumors such as BRCA, LUAD, LUSC, LIHC, and LGG [9][10][11][12][13][14][15]. *NUPR1*, also known as p8, is a transcriptional regulator that has been shown to reduce apoptosis caused by dihydroartemisinin (DHA), sorafenib, or ionizing radiation (IR) in LIHC tumor cells [9][10][11]. However, opposing effects have been observed in osteosarcoma and non-tumor cells [16]. Additionally, research has demonstrated that Δ9-tetrahydrocannabinol (THC) induces autophagy-mediated apoptosis in an LGG model [12]. Despite autophagy-related pathways being upregulated in LIHC and LGG tumors, it remains uncertain whether these pathways promote tumor growth or tumor suppression, necessitating further investigation. In lung and breast cancers (LUAD, LUSC, and BRCA), repression of *NUPR1*, in combination with conventional anticancer therapies, has been proven to control tumor growth [13][14][15]. Another study supports the inhibition of *NUPR1* using microRNA-637 (hsa-miR-637) as a promising option for this purpose [17].

Controversial results have emerged regarding the expression of the *PRKAA2* gene (Protein Kinase AMP-Activated Catalytic Subunit α 2, AMPKα2) in gastrointestinal malignancies [18][19][20][21]. Some studies suggest that repression of *PRKAA2* promotes tumor growth in gastrointestinal cancer by suppressing ferroptosis, an autophagy-dependent form of cell death [18]. On the other hand, other studies propose that *PRKAA2* activates autophagy-related pathways, leading to treatment resistance, and that its activation can be triggered by the gastrin hormone [19][20][21]. In LIHC, inhibiting *PRKAA2* has been shown to downregulate autophagy rates, and metformin has been identified as a potential *PRKAA* agonist for controlling hepatitis C virus (HCV) replication [22]. In glioma, low expression of *PRKAA2* has been associated with a better prognosis [23]. Although these findings may seem contradictory, especially considering that LGG belongs to Cluster "0", they underscore the importance of including autophagy-related factors in the intra- and inter-individual heterogeneity of tumors.

The *MAPT* gene encodes the microtubule-associated protein tau, which has been extensively studied in Alzheimer's disease (AD) [24][25]. Recent research has explored the interplay between autophagy and *MAPT* in AD and has demonstrated that overexpression of *MAPT*/tau inhibits the fusion of autophagosomes with lysosomes, leading to autophagosome accumulation through increased levels of LC3 protein [26]. Although direct links between *MAPT* and autophagy in cancer remain limited, the high expression levels of Tau protein in glioblastoma, a tumor with enhanced autophagy activity, have raised questions about its possible role in oncogenesis and its implications for cancer therapy [27].

In BRCA cohorts, a long non-coding RNA (lncRNA) for the *MAPT* gene called MAPT-AS1 has been found to be overexpressed in tumor tissues [28][29][30]. This is noteworthy because lncRNAs, which are usually located in antisense strands of DNA from original genes, can also be affected by somatic mutations irrespective of their canonical effects. Thus, the combination of somatic mutations and non-coding RNA as potential prognostic markers deserves further attention, as demonstrated in COAD [31].

TP53INP1 gene exhibits inconsistent findings across experiments and tumor tissues. Some researchers have identified hsa-miR-106a as an oncomiR that targets *TP53INP1* in metastatic lung cancer [32], indicating its involvement in tumor suppression. Increasing the levels of *TP53INP1* could be crucial in controlling tumor growth through autophagy-dependent cell death. In the case of PRAD, hsa-miR-30a and hsa-miR-205 have been suggested as potential therapeutic options for suppressing *TP53INP1* [33][34]. However, it has been explained that *TP53INP1* is overexpressed as a response to ionizing radiation, which confers resistance [33][34]. Therefore, suppressing this gene could potentially resensitize tumor cells to standard treatment protocols. Like other representative genes in this cluster, *TP53INP1* exhibits a dual function. According to Peuget et al. (2021), oxidative stress induces the expression of *TP53INP1* [35]. This stress can trigger autophagy by interacting with LC3 in the cytoplasm or apoptosis by interacting with P53 in the nucleus, and the role of mitochondria and their metabolism in this process is also implicated [36]. Thus, an additional factor to consider in researchers' analysis is the localization of autophagy-related transcripts and proteins. Unfortunately, there is insufficient information available to conduct this type of comparison.

In summary, NUPR1, PRKAA2, TP53INP1, ACTL6B, KRBA1, EEF1A2, and MAPT genes are coexpressed with 17 other genes (ANK2, ST8SIA1, GUCY2F, HERC1, TRHR, COL11A1, CHRM3, CNR2, KITLG, ROR1, CDKL5, PPOX, IGF2R, DDIT3, OPCML, ELOVL5, and BRINP2) according to the GeneMania database [37]. These genes are enriched in the MAPK pathway (p = 0.004) [38], allowing us to associate cluster "0" with a MAPK-dependent macroautophagy-like process. However, it is important to note the significant heterogeneity observed in the samples, classifications, tumor tissues, and other forms of autophagy.

3. Tumors Balancing Macro- and Micro-Autophagy Processes (Clusters 0 and 1)

Clusters "0" and "1" in **Figure 1** represent a distinct group of genes associated with tumors that exhibit a balance between macroautophagy and microautophagy processes. Notably, the genes *SREBF1*, *OPTN*, *ACBD5*, *SESN3*,

KERA, *TUBA3D*, *FBXW7*, *TBC1D12*, *TLR9*, and *PLK2* show high expression levels in various tumors such as ESCA, PAAD, STAD, COAD, LUAD, LUSC, KIRC, LGG, PRAD, KIRP, LIHC, and BRCA.

In addition, after performing a random forest Gini importance analysis, researchers observed that KERA, TP53INP1, SREBF1, and TUBA3E showed great accuracy (above 75%) and over 75% of Gini contribution. It suggests the potential contribution of these autophagy-related genes in the classification of tissues regarding their dysregulation between tumor and normal samples.

Of particular interest are the *TUBA3D* and *FBXW7* genes, which are associated with the chaperone-mediated protein folding pathway (R-HSA-390466) according to the Enrichr database [38]. This suggests their potential involvement in chaperone-mediated autophagy. Supporting this idea, these genes have also been implicated in certain forms of microautophagy, such as aggrephagy and mitophagy, as indicated by the MSigDB. Additionally, these genes are part of the regulatory pathways of macroautophagy along with the other eight genes that cluster these tumors. *ACBD5*, *SREBF1*, and *OPTN* genes are also involved in microautophagy pathways, including aggrephagy, mitophagy, and xenophagy.

3.1. Accumulation of ACBD5 Is Found in Tumors from Cluster 0 and 1

Notably, the *ACBD5* gene is interesting in autophagy-related studies as its deregulation can induce their accumulation at protein levels. This gene has been associated with peroxisome maintenance, lipid exchange, and homeostasis, which are crucial processes for lipid and carbohydrate metabolism reorganization in tumor cells [39][40]. These processes involve microautophagy pathways such as pexophagy, aggrephagy, and mitophagy [41].

3.2. Previous Research on Overexpressed Genes in Tumors of Clusters 0 and 1

Other genes related to microautophagy processes include *PLK2*, *SESN3*, *TLR9*, *OPTN*, and *SREBF1*. Independent research has demonstrated that the *PLK2* gene controls α-Synuclein aggregation in an autophagy-dependent context [42]. Although this process is dependent on macroautophagy and regulated by mTORC1 inhibition, it appears to be a microautophagy pathway that is specifically activated in the presence of its substrate, α-Synuclein [42][43]. An interesting regulatory axis involves the lncRNA OIP5-AS1, which targets hsa-miR-126 to prevent α-Synuclein aggregation in autophagy-activated cells [43].

Regarding the *SESN3* gene, recent studies have identified its role as an autophagy activator in tumor cells by repressing mTORC1 [44]. However, this gene has also been associated with other autophagy pathways such as chaperone-mediated autophagy [45]. Overexpression of *SESN3* has been observed in LUAD [45] and ESCA [46] models, suggesting its potential involvement in promoting pro-tumor autophagy pathways. Expression levels of this gene can be regulated by specific miRNAs, such as hsa-miR-194-3p [45] or hsa-miR-429 [46].

About mitophagy, several reports have described the upregulation of the *TLR9* gene in tumors belonging to Clusters “0” and “1” [47][48][49], indicating its involvement in inducing this form of autophagy. In BRCA, it has been reported that this gene plays a role in the rewiring of doxorubicin and may explain the cardiomyocyte death and

systolic dysfunction observed in patients undergoing this tumor treatment [50]. Consistent with these findings, *TLR9* was found to be upregulated in aggressive versions of LIHC, LUAD, LUSC, and COAD models [51][52][53]. Consequently, various regulatory pathways have been proposed to control *TLR9* expression. For example, hsa-miR-30a has been shown to sensitize LIHC cells to a combined therapy of hydroxychloroquine and sorafenib by repressing *TLR9* [51]. On the other hand, inducing *TLR9* expression in dendritic cells has been suggested as a potential therapeutic strategy, as demonstrated in PAAD cases [54]. It is important to note that bulk analyses using next-generation sequencing (NGS) do not differentiate between the origins of cells within tumors, which can lead to different interpretations of the results. Therefore, researchers are increasingly turning to single-cell sequencing to differentiate immune cells, tumor cells, and normal-adjacent cells with varying autophagy-related profiles within the same tumor pool.

In addition to *TLR9*, *OPTN* has been extensively studied in the context of mitophagy. *PINK1* and *PRKN*, which are highly studied autophagy-related genes, are also involved in this process. The *PHB2* gene stabilizes *PINK1* in mitochondria, facilitating the recruitment of Parkin (the product of *PRKN*), ubiquitin, and optineurin (the product of *OPTN*) to promote mitophagy [55][56][57][58]. However, a recent study challenges the necessity of *PINK1* and *PRKN* for initiating mitophagy [59]. Consequently, it has been suggested that *OPTN* may have tumor suppressor functions by activating suppressor autophagy mediated by *HACE1*, a tumor suppressor [60][61][62], or by repressing the pro-oncogenic transforming growth factor-β (TGFβ) signaling in triple-negative breast cancer (TNBC) cells, a subtype of BRCA [63]. Importantly, *OPTN* has been found to be downregulated in GBM tumor samples, which has been corroborated by independent studies [64]. The same study proposes that inducing *OPTN* expression in GBM cells could help control tumor growth, supporting a suppressive role for this gene, although the underlying mechanism remains unknown.

In terms of the application of *OPTN* in the context of mitophagy and the tumor environment, several studies have identified *OPTN* as a potential therapeutic target. For instance, it has been observed that *OPTN* induces pro-tumor mitochondrial-related autophagy, reducing the efficacy of combined treatments involving pemetrexed, cisplatin, and MEK inhibitors or anti-PD-L1 in a LUSC model [52]. In a PAAD model, repression of *OPTN* leads to apoptosis through chaperone-mediated autophagy [65]. Similar to *TLR9*, understanding the function of *OPTN* allows us to differentiate its contribution to tumor growth based on its expression in surrounding cells. In LUAD models, higher expression of *OPTN* in fibroblasts surrounding the tumor contributes to tumor invasiveness [66].

SREBF1 upregulation has been linked to mTORC1-dependent autophagy, which may be induced by leptins to suppress ferroptosis in BRCA, LIHC, PRAD, and LUAD models [67][68][69][70]. Additionally, *SREBF1* levels were found to be elevated in PAAD tissues, regulated by high glucose concentrations. In PAAD models, the upregulation of *SREBF1* helps control autophagy levels [71]. This gene may act as a negative regulator of mTORC1-dependent autophagy, favoring pro-tumor microautophagy pathways. It is worth noting that *SREBF1* can function as both a protein and a transcription factor. Studies have demonstrated that genes upregulated by the *SREBF1* transcription factor can be altered in the presence of cisplatin, inducing treatment resistance in a LUSC model [72]. This evidence highlights the importance of carefully analyzing autophagy-related genes with dual functions to enhance researchers' understanding of this process. A study proposed that mTORC2 stabilizes *SREBF1* through *FBXW7-*

mediated regulation to integrate autophagy and lipid metabolism processes, leading to the downregulation of target genes such as acetyl-CoA carboxylase and fatty-acid synthase [73].

Considering the combined findings of two genes involved in tumor clusterization, *FBXW7* and *SREBF1*, it is hypothesized that these tumors exchange autophagy-related processes and large-scale technologies based on their aggressiveness and treatment sensitivity or resistance. However, conducting large-scale high-throughput analyses in mass groups could obscure specific autophagy pathways in certain tumor subtypes or patients. Therefore, the current perspective is to compare global observations with focused research. Nevertheless, the scientific community is moving towards a comprehensive analysis of tumors, considering their heterogeneity and subclonal profile, which will allow us to confirm researchers' current hypotheses about autophagy-related processes in the tumor environment in the future.

Regarding macroautophagy, the *FBXW7* gene has been the focus of numerous studies aiming to characterize its function. This gene is known as a tumor suppressor as it is frequently mutated or suppressed in human tumors [74]. However, its dysregulation in chemoresistance remains controversial, suggesting that its behavior depends on the context. It has been observed to be upregulated in resistant gastric cells [75] and downregulated in chemoresistant models of BRCA [76].

Interestingly, *FBXW7* has been found to induce the expression of *ATG16L1*, an important gene involved in LC3 lipidation and autophagosome formation, while not affecting the levels of other autophagy-related genes (ATG) [77]. Moreover, *FBXW7* suppresses mTORC1, thereby activating autophagy pathways [78][79]. *FBXW7* participates in different molecular axes, resulting in different effects on tumor cells. For instance, the GSK3-FBXW7 interaction leads to the ubiquitination and degradation of Rictor, increasing cellular ROS (reactive oxygen species) in an autophagy-activated context [80]. On the other hand, interactions between *FBXW7* and oncogenes such as *SHOC2* or *LSD1* can reduce the expression of autophagy-related pathways [78][79][81]. In conditions where tumors are growing, cisplatin treatments have been shown to induce the degradation of the MRE11-RAD50-NBS1 (MRN) complex by *FBXW7* and lysosomes [74]. As a result, the overexpression of the MRN complex or the suppression of the *FBXW7* gene can lead to cisplatin-resistant tumors and a poor prognosis. In relation to this, hsa-miR-25 and hsa-miR-223 have been shown to suppress *FBXW7* levels, promoting autophagy and treatment resistance in LIHC [82] and LUAD [83] models, respectively. Anti-miRs could be used to counteract the suppression of *FBXW7* levels, but it is important to better understand the specific context in which this strategy would be applicable.

Lastly, three genes (*TBC1D12*, *KERA*, and *TUBA3D*) that contribute to tumor clustering in groups “0” and “1” have not been previously associated with the tumor-related autophagy process. It is important to emphasize that, in researchers' analysis, the *KERA* gene was the top gene in Gini relevance and accuracy in tissue pooling of groups between 0 + 1 vs. 2. Studies on mutations in the *TBC1D12* gene (TBC1 Domain Family Member 12) have been conducted in urological tumors, suggesting that alterations in its mutational profile could be linked to worse patient survival [84]. Interestingly, this gene exhibits a higher mutation frequency in PRAD samples compared to other patients. The *KERA* (Keratan Sulfate Proteoglycan Keratocan) gene has been found to have lower levels in cisplatin and paclitaxel-resistant OV models [85], partially aligning with observations in the entire dataset (Cluster

"2"). The expression levels of the *TUBA3D* (Tubulin α-3D Chain) gene in BRCA (upregulated) and OV (downregulated) have been validated [86][87]. Notably, in BRCA models, *TUBA3D* was shown to be downregulated in paclitaxel-resistant cells compared to parental cells [88].

In summary, the findings presented in this discussion suggest that all the aforementioned genes may make significant contributions to tumor-related autophagy through their expression in tumors and the surrounding cells, warranting further attention in future research.

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