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BIOTECHNOLOGY

Schwann cell reprogramming and Esophageal squamous cell carcinoma: analysis of transcriptome data

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ABSTRACT. Perineural invasion (PNI) occurs when cancer cells infiltrate nerves or the adjacent nervous tissue. The significance of PNI in Esophageal cancer remains controversial. Therefore, identifying new prognostic factors for Esophageal squamous cell carcinoma (ESCC) is important for optimizing treatment and improving outcomes. We investigated transcriptomic datasets and differentially expressed genes (DEGs) through meta-analysis and bioinformatics analysis. The set of core DEGs was subjected to functional and pathway enrichment analyses and integrated with genome-scale human biomolecular networks. We also analyzed how each gene was correlated with patient survival. Our findings indicate that the "cGMP-PKG signaling pathway" and "Cytokine-cytokine receptor interaction" pathways were deregulated in ESCC. These pathways play a role in the regeneration of Schwann cells and the progression of ESCC. Also, different expressions of NCAM1 and s100 genes suggest that Schwann cells are involved in this type of cancer's PNI. Our results suggest that the tumor can induce the dedifferentiation of Schwann cells, which promotes detachment of cancer cells from the tumor, guiding them to the perineural tissue. This study's results encourage further studies for clinical and experimental validation of the data found.

Keywords: Esophageal neoplasms; esophageal squamous cell carcinoma; Schwann cells; computational biology.

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Introduction

Perineural invasion (PNI) occurs when cancer cells infiltrate nerves or the adjacent nervous tissue. This process can be observed before vascular and lymphatic invasion (Wang et al., 2021; Batsakis, 1985; Liebig, Ayala, Wilks, Berger, & Albo, 2009; Magnon et al., 2013; Tan et al., 2021). PNI is most reported in head and neck cancers, with an incidence as high as 80%. Such a phenomenon is considered a significant risk factor for invasion and metastasis, and is therefore regarded as a negative prognostic factor (Saloman, Albers, Rhim, & Davis, 2016). However, the significance of PNI in Esophageal cancer remains controversial; while some studies suggest that PNI status is related to a poor prognosis in esophageal squamous cell carcinoma (ESCC), others question its role as a predictor of prognosis (Saloman, Albers, Rhim, & Davis, 2016; Liebig et al., 2009; Huang et al., 2014; Hibi et al., 2009). Therefore, identifying new prognostic factors for ESCC in addition to the current staging system is important for optimizing treatment and improving outcomes.

Nerves and cancer cells stimulate each other to promote growth (Ayala et al., 2021; Deborde, & Wong, 2017). Schwann cells are a major component of the peripheral nerves and have been recently identified as cells that encourage spread of cancer (Deborde, & Wong, 2017). Cancer cells are recruited to neurites and migrate along the nerve fiber toward the dorsal root ganglia (DRG) center (Ayala et al., 2021; Deborde, & Wong, 2017). Recently, time-lapse recordings have demonstrated cancer cell migration along neurites and the active participation of Schwann cells in recruiting cancer cells to neurites (Deborde, & Wong, 2017; 12). The study of the mechanisms of PNI has led to the understanding that the nerve is not only a preferred location for cancer cells, but also that it promotes cancer initiation and tumor growth.

Schwann cells trigger cancer invasion by using specific cellular mechanisms (Deborde, & Wong, 2017). In-vitro studies have shown that Schwann cells can degrade the matrix around cancer cells, stimulating cancer cell protrusion after contact and guiding cancer cells during invasion (Deborde, & Wong, 2017,12). Schwann cells interact with cancer cells through a variety of signaling pathways. These interactions facilitate the migration and invasion of cancer cells along nerves. Schwann cells secrete chemotactic factors, such as nerve growth factor (NGF) and glial cell line-derived neurotrophic factor (GDNF), which attract cancer cells to nerves and promote their invasion (Wang et al., 2021; Saloman, Albers, Rhim, & Davis, 2016; Kuol, Stojanovska, Apostolopoulos, & Nurgali, 2018). In addition to their signaling role, Schwann cells modify the microenvironment in ways that promote PNI. They produce matrix metalloproteinases (MMPs), which degrade the extracellular matrix (ECM) and create pathways for cancer cells to invade. Furthermore, Schwann cells can modulate the immune response, creating an immunosuppressive environment that allows cancer cells to evade immune detection and destruction (Wang et al., 2021; Deborde et al., 2016). Direct physical interactions between Schwann cells and cancer cells also facilitate PNI. These interactions involve cell adhesion molecules that help guide cancer cells along nerve fibers. Schwann cells also provide trophic support to cancer cells, enhancing their survival and promoting their invasive capabilities (Saloman, Albers, Rhim, & Davis, 2016; Deborde, & Wong, 2017; Kuol, et al, 2018).

To investigate this process, we studied transcriptomic datasets and differentially expressed genes (DEGs) through meta-analysis and bioinformatics analysis. The set of core DEGs was subjected to functional and pathway enrichment analyses, and was integrated with genome-scale human biomolecular networks. Data on DEGs were obtained from the Gene Expression Omnibus (GEO) and The Cancer Genome Atlas (TCGA), which are publicly available databases. We also analyzed how each gene was correlated with patient survival. As a result, this study presents possible biomolecules to be explored as biomarkers or potential therapeutic targets in future clinical and experimental trials for PNI risk in patients with ESCC.

Materials and methods

Collection and criteria for inclusion of studies

The TCGA database (https://portal.gdc.cancer.gov/) was used to obtain gene expression profiles and clinical data of patients with ESCC. The samples included microRNA sequencing and RNA sequencing data. As this study used only public domain data from the TCGA, it is not possible to identify the subjects. Thus, approval by the Ethics Committee was no longer necessary.

Microarray data and data processing

The GEO database was used to download the GSE4030 gene expression profile. DEGs in human Schwann cells from early and late passage exposed to cancer growth factors were identified using the GEO2R (http://www.ncbi.nlm.nih.gov/geo/geo2r/). Considering that both groups in this comparison were exposed to mitogens, differences in gene expression profiles will be interpreted as indicative of changes caused by prolonged versus short-term exposure. Benjamini and Hochberg's false discovery rate method by default was used to correct false-positive results. DEGs were identified based on statistical significance, typically using an adjusted p-value (FDR) cutoff of less than 0.05. Additionally, the magnitude of expression change was considered, with genes showing a fold change greater than or equal to 1 (upregulated) or less than or equal to -1 (downregulated) being selected. Consistency of expression across multiple samples or replicates was also a criterion to minimize the impact of outliers or experimental noise.

Gene set collection

Entrez Gene from National Center for Biotechnology Information (NCBI) (www.ncbi.nlm.nih.gov/gene/) and GeneCards (https://www.genecards.org/) were used as identifiers for genes related to Schwann cell dedifferentiation. The list of genes and downregulated/upregulated proteins from previously published data (Tenze et al., 2016) were combined and identified with a Venn diagram 2.1.0 (http://bioinfogp.cnb.csic.es/tools/venny/index.html).

Functional and pathway enrichment analysis

The evolutionary relationships program PANTHER (www.pantherdb.org) was used to analyze the genetic ontology (GO) of proteins, which includes their cellular components, molecular functions, and relevant biological processes. GO terms assigned into identified molecules were classified according to their functions (Bastos, Tavares, Pesquita, Faria, & Couto, 2011). The Kyoto Encyclopedia of Genes and Genomes (KEGG), a database generated with molecular information for genome sequencing and polymer experiment technology, was used to predict gene enrichment pathways (Kanehisa, Sato, Kawashima, Furumichi, & Tanabe, 2016). KEGG analyses were available in the DAVID database (https://david.ncifcrf.gov/), a resource used to extract important biological information from many genes and proteins. Within DAVID, KEGG pathway enrichment analysis is performed to determine which pathways are significantly overrepresented among the DEGs. The analysis employs Fisher's Exact Test to calculate the significance of the association between the DEGs and the KEGG pathways. An adjusted p-value (FDR) of less than 0.05 is used to account for multiple testing and ensure the robustness of the results. DAVID also provides a functional annotation clustering feature, which groups enriched pathways based on their biological themes. This clustering helps in understanding the broader biological processes and interactions in which the DEGs are involved (Huang, Sherman, & Lempicki, 2009).

RNA-seq and clinical information data from The Cancer Genome Atlas (TCGA)

Genomic and clinical data on normal tissues and ESCC tumor tissues were downloaded from TCGAbiolinks, a R/Bioconductor software (http://bioconductor.org/packages/release/bioc/html/TCGAbiolinks.html) (Colaprico et al., 2016), and from the interface TCGAbiolinksGUI (Silva et al., 2018). The recovered data were applied to raw count mRNA and miRNA expression (Illumina HiSeq 2000). The Venn Diagram 2.1.0 (http://bioinfogp.cnb.csic.es/tools/venny/index.html) was applied to combine upregulated and downregulated DEGs co-expressed in gene expression profiles. An adj. $P < 0.05$ and a $\log FC \geqslant 1$ were defined as the cut-off criteria.

KM-Plotter

The Kaplan-Meier Plotter (KM-Plotter (http://kmplot.com/analysis/) is a database generated with gene expression and survival information obtained from GEO, commonly used to analyze the impact of genes on relapse-free survival (RFS), distant metastasis-free survival (DMFS), overall survival (OS) and post-progression survival (PPS) in patients with different types of cancer. In this study, KM-Plotter was used to investigate the role of mRNA expression in the prognosis of ESCC.

Results

Identification of gene ontology enrichment, differentially expressed genes, and functional classification

We extracted gene expression data on ESCC samples from the TCGA, out of which only the ESCC subtype was selected. The data were preprocessed using the standard procedure. When identification of DEGs was finished, a DAVID analysis was performed using them. Downregulated genes in ESCC belong to "Gastric acid secretion", "Dilated cardiomyopathy" and "Calcium signaling pathway" pathways. While "Cell cycle" and "DNA replication" were the most active pathways in ESCC (Tables 1–2).

Overview of the cancer transcriptomic analysis

We conducted a systematic and integrative analysis to explore type-specific cancer and Schwann cell-specific DEGs to construct a cancer network. Initially, we determined DEGs by comparing gene expression levels between tumor and normal samples. A Venn diagram was then constructed to visualize the overlap between DEG genes, both upregulated and downregulated, from both cancer types and Schwann cells. Results are shown in Figure 1. Among downregulated genes from ESCC, 107 DEGs co-expressed by Schwann cells were found. Regarding upregulated genes, 42 were identified.

A KEGG analysis was performed to investigate pathways with major expression changes in studied cell lines and ESCC, based on previously identified overlapping DEGS. The analysis showed that the "cGMP-PKG signaling pathway" was the only significant pathway concerning overlapped downregulated genes from Schwann cells and ESCC DEGs. In contrast, as for upregulated genes, overlapped Schwann cells and ESCC DEGs samples deserve to be highlighted. "Cytokine-cytokine receptor interaction" and "Measles". *KCNJ8, PDE3A, PDE5A, MRVI1, CACNA1C,* and *SLC8A1* were the commonly downregulated genes associated with the "cGMP-PKG signaling pathway" pathway; *TNFRSF18, IL1B, TNFRSF9, IL2RA,* and *IL12RB1* were frequently associated with the "Cytokine-cytokine receptor interaction" pathway.

Analysis of genes involved in dedifferentiation of Schwann cells

To understand the mechanism by which Schwann cells aid in neoplastic development, we analyzed the behavior of genes associated with cell differentiation maintenance proteins (*SOX10, S100, EGR2, MBP,* and *MPZ*). With this mechanism, the expression of a new set of proteins that form non-myelinated cells, SOX10, GAP43, S100, NCAM1, NGFR1, and GFAP, is augmented. While some genes from the s100 family (*S100A3, S100A6, S100A7, S100A7A, S100A10, S100A11*) showed an increased mRNA expression in ESCC samples, only NCAM1 protein expression was decreased.

Prognostic analysis

A survival analysis of the core genes was conducted. The analyses showed that *MSH2, EXO1, PER1, MYB, CCNE2, TNFRSF18, ITGA2, TARC1, RFC3, RPS6KA5, CDK2, BRCA1, RFC5, ANGPT2, ITGB8, CDK4,* and *POLD3* lower expressions are associated with poor ESCC prognosis (Figure 2). However, a KM Plotter analysis demonstrated that *EFNA1, S1PR1, GNAO1, LEPR, HRH2, PLCL1, NPR1, PDE1B, IL12RB1, PDE1A, GNG7, TACR2,* and *PIK3AP1* higher expressions are associated with lower survival rates in ESCC samples (Figure 3).

Discussion

We investigated the association between Schwann cells and ESCC. The GEO data platform from the GEO repository was used to search for a dataset that provides information about Schwann cells' expression in a neoplastic scenario. The dataset has experimental information about the gene expression of Schwann cells exposed to growth factors released by neoplastic cells. After analyzing the DEGs of Schwann cells in a tumor microenvironment, we compared the data with the DEGs of ESCC. An analysis to identify the molecular pathways associated with overlapping genes was performed. The downregulated genes were involved in the "cGMP-PKG signaling pathway", and the upregulated genes were associated with "Cytokine-cytokine receptor interaction" and "Measles" pathways. Studies have demonstrated that the "cGMP-PKG signaling pathway" is involved in the injury and repair mechanisms of Schwann cells and participates in the development and proliferation of ESCC (Zhou et al., 2017; Liu et al., 2020).

The pathway "Cytokine-cytokine receptor interaction" is important for the regulation of neuroprotection and regeneration, also, it affects Schwann cells' myelination and is involved in ESCC metastasis (Yi et al., 2017; Okuda, et al, 2017). Genes such as *TNFRSF18, IL1B, TNFRSF9, IL2RA,* and *IL12RB1* were frequently associated with the "Cytokine-cytokine receptor interaction" pathway. Cytokine signaling is crucial for immune response and inflammation, and its dysregulation is often observed in cancer, promoting tumor growth and immune evasion. The involvement of these pathways underscores the complex interplay between cancer cells and the immune system, highlighting potential targets for therapeutic intervention (Okuda, Inoue, Fujiwara, Kawano, & Inazawa, 2017).

The downregulated genes in ESCC, identified through DAVID analysis, belong to pathways such as "Gastric acid secretion," "Dilated cardiomyopathy," and "Calcium signaling pathway." These pathways are crucial in various physiological processes and their disruption can contribute to cancer progression (Okuda et al., 2017). For instance, the calcium signaling pathway plays a significant role in cellular processes such as proliferation, differentiation, and apoptosis. Disruption of this pathway can lead to uncontrolled cell growth, a hallmark of cancer. The involvement of the "Dilated cardiomyopathy" pathway suggests potential metabolic and structural changes in cancer cells, reflecting their ability to adapt to new environments and sustain growth. In contrast, the "Cell cycle" and "DNA replication" pathways were identified as the most active pathways in ESCC, indicating their upregulation. These pathways are essential for cell division and DNA synthesis, and their activation is a common feature in cancer cells, enabling rapid proliferation (Yi et al., 2017). The upregulation of these pathways supports the aggressive nature of ESCC, contributing to its progression and poor prognosis.

PNI has been associated with an important mechanism of growth, spread, and metastasis of malignant tumors, including pancreatic, prostate, colon, and head and neck cancers. Nonetheless, it has been related to higher recurrence and lower survival rates (Liebig et al., 2009; Bapat, Hostetter, Von Hoff, & Han, 2011). PNI is promoted by Schwann cells, which, after being chemoattracted to the tumor's microenvironment, promote the detachment of cancer cells, which migrate to the nerves (Demir et al., 2014; Deborde et al., 2016). Schwann cells' ability to invade the tumor microenvironment and promote perineural invasion with cancer cells is linked to the expression of myelinating differentiation proteins, enabling Schwann cells to lose their specialized functions and then regain them after nerve injury (Deborde et al., 2016; Jessen, Mirsky, & Lloyd, 2015). In this study, the expression of proteins that form non-myelinated cells, such as SOX10, GAP43, S100, NCAM1, NGFR1, and GFAP is augmented, indicating a shift towards a more proliferative and less differentiated state. This dedifferentiation may facilitate the interaction between Schwann cells and cancer cells, promoting perineural invasion.

S100 protein family plays an important role in cancer progression and is involved in the growth, differentiation, invasion, and migration of cancerous cells (Chen, Xu, Jin, & Liu, 2014). In ESCC, the downregulation of S100A2, S100A8, S100A9, and S100A14, and the upregulation of S100A4 were associated with poor tumor differentiation (Chen et al., 2013; Zhang et al., 2012; Cao, Yin, Li, Jiang, & Zhang, 2009; Kong et al., 2004). However, the increase of the s100 protein family, associated with dendritic and Langerhans cell invasion, has been correlated with a better prognosis in two previous studies. These proteins and the dendritic cells act in the antigens' presentation, an anti-tumor defense mechanism (Matsuda et al., 1990; Furihata et al., 1992).

NCAM proteins expressed in cancers with PNI act in cell adhesion and tumor invasion (Chen et al., 2019). After invading the tumor's environment, the Schwann cells induce cancer cells to express NCAM1, a cell adhesion protein that facilitates the migration of tumor cells toward Schwann cells and nerve invasion (Deborde et al., 2016) demonstrated that the depletion of NCAM1 in the tumor's microenvironment led to a decrease in the ability of Schwann cells to induce adhesion and formation of protrusions from cancer cells, necessary for PNI. NCAM1 is also involved in orienting tumor cells towards the nerve because when the Schwann cell retracts, the cancer cell remains connected to it through NCAM, following it toward the perineural tissue (Deborde et al., 2016).

Then, we analyzed which hub genes were associated with poor ESCC prognosis. We found that higher expressions of *EFNA1, S1PR1, GNAO1, LEPR, HRH2, PLCL1, NPR1, PDE1B, IL12RB1, PDE1A, GNG7, TACR2,* and *PIK3AP1* are associated with lower survival rates in ESCC samples. A previous study showed that the overexpression of *EFNA1* is associated with ESCC poor prognosis (Chen, Zhang, Peng, Chen, & Cui, 2019). Liu et al. (2019) demonstrated that the upregulation of *S1PR1* promotes proliferation and inhibits apoptosis of esophageal cancer cells. Contrary to our results, Ohta et al. (2008) found that the downregulation of *GNG7* was associated with aggressivity and lower survival rates. Mutations in *PIKCA3* in benign lesions play a role in the development of ESCC, as well as being associated with lower survival (Munari et al., 2018).

Abbreviations

DEGs: differentially expressed genes
DMFS: Distant metastasis-free survival
ESCC: Esophageal squamous cell carcinoma
GEO: Gene Expression Omnibus
GO: Genetic ontology
KEGG: Kyoto Encyclopedia of Genes and Genomes
KM-Plotter: Kaplan-Meier Plotter
OS: Overall survival
PNI: Perineural invasion
PPS: Post-progression survival
RFS: Relapse-free survival
TCGA: The Cancer Genome Atlas

Conclusion

In summary, we noticed that the "cGMP-PKG signaling pathway" and "Cytokine-cytokine receptor interaction" pathways were deregulated in ESCC. These pathways play a role in the regeneration of Schwann cells and the progression of ESCC. Also, the different expressions of *NCAM1* and *s100* genes suggests that Schwann cells are involved in this type of cancer's PNI. Our findings suggest that the tumor can induce the dedifferentiation of Schwann cells. Thus, promoting the detachment of cancer cells from the tumor, guiding them to the perineural tissue. Our results encourage further studies for clinical and experimental validation of the data found.

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