Differential Gene Expression in Patients With Prostate Cancer and in Patients With Parkinson Disease: an Example of Inverse Comorbidity

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Abstract

Prostate cancer (PCa) is one of the leading causes of death in Western countries. Environmental and genetic factors play a pivotal role in PCa etiology. Timely identification of the genetic causes is useful for an early diagnosis. Parkinson's disease (PD) is the most frequent neurodegenerative movement disorder; it is associated with the presence of Lewy bodies (LBs) and genetic factors are involved in its pathogenesis. Several studies have indicated that the expression of target genes in patients with PD is inversely related to cancer development; this phenomenon has been named "inverse comorbidity". The present study was undertaken to evaluate whether a genetic dysregulation occurs in opposite directions in patients with PD or PCa. In the present study, next-generation sequencing (NGS) transcriptome analysis was used to assess whether a genetic dysregulation in opposite directions occurs in patients with PD or PCa.

The genes *SLC30A1*, *ADO*, *SRGAP2C*, and *TBC1D12* resulted up-regulated in patients with PD compared to healthy donors as controls and down-regulated in patients with PCa compared with the same control group.

These results support the hypothesis of the presence of inverse comorbidity between PD and PCa.

Introduction

In the United States and Europe, prostate cancer (PCa) is the leading cause of cancer-related death among men [1, 2]. The prognosis is good for patients with localized or regional disease; nevertheless, the survival average in patients with metastasis is only 12–15 months [3–5]. The exact mechanisms involved in PCa initiation and progression have not been clearly understood yet, although genetic factors are believed to play a role [6].

Parkinson's disease (PD) is the most frequent neurodegenerative movement disorder, which is histologically featured by neuronal loss (specifically occurring in the *substantia nigra*) and the by the presence of Lewy bodies (LBs) [7, 8]. On a clinical point of view, PD is characterized by the onset of specific motor symptoms (e.g. akinesis, rigidity, postural instability and resting tremor), which are due to the progressive dopaminergic nigrostriatal system degeneration. PD is a very complex disorder. It indeed involves multiple biochemical deficits that are extended to the peripheral nervous system. For this reason, PD had been recently redefined as a multisystem disorder with a genetic background [9].

Epidemiological data have shown that patients with central nervous system (CNS) disorders, including PD, have a lower susceptibility to develop cancer [10–13]. This has been defined "inverse comorbidity". More specifically, inverse comorbidity is a lower-than-expected probability that a disease will occur in people who have other diseases. Emerging evidence indicates lower tumor comorbidity in patients with some CNS disorders including PD [11]. Such inverse comorbidity is influenced by environmental, pharmacological, and alimentary factors. However, genetic factors can additionally contribute to the inverse comorbidity between complex diseases [10–13]. Accordingly, several studies have indicated that

the expression of target genes in patients with PD is inversely related to tumor development [14–16]; nevertheless, no data are available on the possible presence of inverse comorbidity between PCa and PD. Therefore, the present study was undertaken to evaluate whether a genetic dysregulation occurs in opposite directions in patients with PD or PCa. To accomplish this, next-generation sequencing (NGS) transcriptome analysis.

Results

NGS transcriptome analysis showed that the genes *Solute Carrier Family 30 Member 1* (*SLC30A1*), *2-Aminoethanethiol Dioxygenase* (*ADO*), *SLIT-ROBO Rho GTPase Activating Protein 2C* (*SRGAP2C*), and *TBC1 Domain Family Member 12* (*TBC1D12*) were over-expressed (adjusted $p < 0.05$) in patients with PD compared to controls (Fig. 1), whereas they were down-expressed in patients with PCa compared to controls (Fig. 1).

These data were further confirmed by RNA sequencing data from enriched prostate CTCs from clinical blood specimens. In particular, raw data generated by Wong and colleagues [17] were reanalyzed as described above and compared to the samples investigated in-house. By analyzing the expression level of the selected four deregulated mRNAs in our patients with PCa ($|FC| \geq 1.5$ and adjusted $p \leq 0.05$), we observed a direct correlation in the two PCa datasets and an inverse correlation with the PD dataset (Fig. 1).

Discussion

SLC30A1, also known as *ZnT1*, is widely expressed in human tissues and primarily localized in the plasma membrane. It contributes to cytoplasmic zinc (Zn) balance by exporting Zn to the extracellular space [18]. Singh and colleagues [19] reported a significant upregulation of *SLC30A1* mRNA in PCa tissue, compared to benign prostatic tissue. Increased expression of *SLC30A1* was also found in patients with Alzheimer's disease [20].

ADO has a role in cysteine metabolism and is localized in the mitochondria. This metabolic pathway is responsible for the synthesis of glutathione with antioxidant properties and is involved in the pathogenesis of various disorders [21]. Sarkar and colleagues [22] highlighted the role of an altered expression and functioning of the *ADO* gene in the pathogenesis of neurodegenerative disorders and cancer [22].

SRGAP2C is involved in the development of the cerebral cortex [23, 24]. The gene encodes for a highly conserved protein that is expressed early in development. More in detail, this protein acts as a regulator of neuronal migration and differentiation by inducing filopodia formation, branching of neurons, and neurite outgrowth [23, 24].

TBC1D12 is a Tre2-Bub2-Cdc16 (TBC) domain family of proteins that colocalize with transferrin receptor (TfR), a well-known recycling endosome marker. The possible role of *TBC1D12* has been highlighted in

several cellular events, including transferrin (Tf) recycling, neurite outgrowth, and endosomal trafficking [25].

Noteworthy, *SLC30A1, ADO, SRGAP2C*, and *TBC1D12* genes play crucial roles in the molecular mechanisms involved in oncological and neurodegenerative processes, as shown in Fig. 2, which summarizes their Biological Process networks. Catalá-López and colleagues [26] showed that the presence of CNS disorders is associated with a reduced co-occurrence of cancer [26]. The concept that a specific disease is able to offer protection against cancer can be traduced in a novel model of inverse cancer morbility, in patients with CNS diseases. Particularly, epidemiological data support the occurrence of a lower-than-expected incidence of cancer in patients suffering from PD, Down's syndrome, diabetes mellitus, Alzheimer's disease, and multiple sclerosis [27]. This knowledge represents an opportunity to deepen CNS pathogenesis. Further studies are needed to understand the reason why specific CNS disorders have a low risk of cancer development. This data may also help in the development of new and more effective therapeutic strategies in the next future.

Conclusion

In conclusion, the data of the present study support the existence of inverse comorbidity between PCa and PD and may represent the starting point to design more in-depth and well-sized studies to confirm these findings.

Materials And Methods

Patient selection

A total of 27 men were included in this study; 7 controls, 13 patients with PD, and 7 with PCa. For the NGS experiments, we recruited 23 participants, including 7 controls with an age range of 54-87 years, 13 patients with PD with an age range of 50-88 years, and 3 patients with PCa aged 54, 71, and 72 years, respectively. Moreover, data deposited by Wong and colleagues [17] on a case history of 4 patients with PCa were analyzed.

Patients with PD and controls were recruited at the Oasi Research Institute-IRCCS, Troina (Italy), whereas patients with PCa were recruited at the Urology Unit, Cannizzaro Hospital, Catania (Italy). Family history was collected through a detailed interview of the wife or the first-degree relatives. The present and past clinical history of each patient was gathered and all available medical records (e.g., drug prescriptions, medical records, certificates) were carefully evaluated. All the diagnoses of PD were made by an experienced neurologist, according to the current PD diagnostic criteria [28]. PCa diagnoses were performed after radical prostatectomy and histological analysis. The diagnosis and grading of PCa were made according to the 2005 International Society of Urological Pathology (ISUP) Consensus Conference [29].

All patients and controls or their relatives gave their informed consent for study participation. The study was carried out in accordance with the Declaration of Helsinki of 1964 and its later amendments, and the Ethics Committee of the Oasi Research Institute–IRCCS, Troina (Italy) approved the protocol on June 03, 2017 (2017/05/31/CE-IRCCS-OASI/9).

RNA extraction

PBMCs separation was performed using Ficoll-Paque (Ficoll Plaque PLUS – GE Healthcare Life Sciences, Piscataway, USA) and the RNA was extracted using TRIzol reagent (TRIzol Reagent, Invitrogen Life Technologies, Carlsbad, CA, USA), according to the manufacturer's instructions [30]. The RNA obtained was stored at -80^{o} C.

RNA sequencing and data analysis

RNA sequencing was performed by Genomix4Life Srl (Baronissi, Italy). RNA concentration in each sample was assayed with an ND-2000c spectrophotometer (NanoDrop) and its quality was evaluated with the TapeStation 4200 (Agilent Technologies). Indexed libraries were prepared from 1 µg/ea purified RNA with TruSeq Stranded Total RNA (Illumina) Library Prep Kit according to the manufacturer's instructions. Libraries were quantified using TapeStation 4200 (Agilent Technologies) and pooled such that each index-tagged sample was present in equimolar amounts, with a final concentration of the pooled samples of 2 nM. The pooled samples were subject to cluster generation and sequencing using an Illumina NextSeq 500 System (Illumina) in a 2x75 paired-end format. The raw sequence files generated (fastq files) underwent quality control analysis using FastQC (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/). Subsequently, the quality-checked reads were trimmed with cutadapt v.1.10 and then aligned to the human genome (hg38 assembly) using STAR v.2.5.2 [31], with standard parameters. The read-count for the genes of interest was computed using feature Counts and normalized using negative binomial generalized linear models, considering all genes expressed in the samples, by Bioconductor DESeq2 package [31,32]. Transcripts showing fold-change ≤ -1.35 or ≥ 1.35 ($|FC| \geq 1.35$), with adjusted *p*-values ≤ 0.05 (padj) were considered as differentially expressed. Gene Ontology (GO) annotation analysis was performed using GO net web-application [33]. The sequencing data are available upon request, as they are partial data of a larger research project not yet completed.

RNA sequencing data from enriched prostate circulating tumor cells from clinical blood specimens

Raw data of enriched prostate circulating tumor cells (CTCs) from 12 (3 blood samples for each patient) clinical blood specimens were downloaded by NCBI GEO (accession number GSE104209) [17]. RNA sequencing data were analyzed as described above and compared to the in-house control.

Declarations

Conflict of interests

The authors declare that they do not have any conflict of interests.

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Authorship contribution statement

Michele Salemi, Pietro Pepe, Rossella Cannarella, Aldo E. Calogero, Giovanna Marchese, Maria Ravo, and Raffaele Ferri planned the study. Michele Salemi, Simona Vetrano, Giovanna Marchese, Maria Ravo, performed experiments and analysed data. All authors edited the manuscript.

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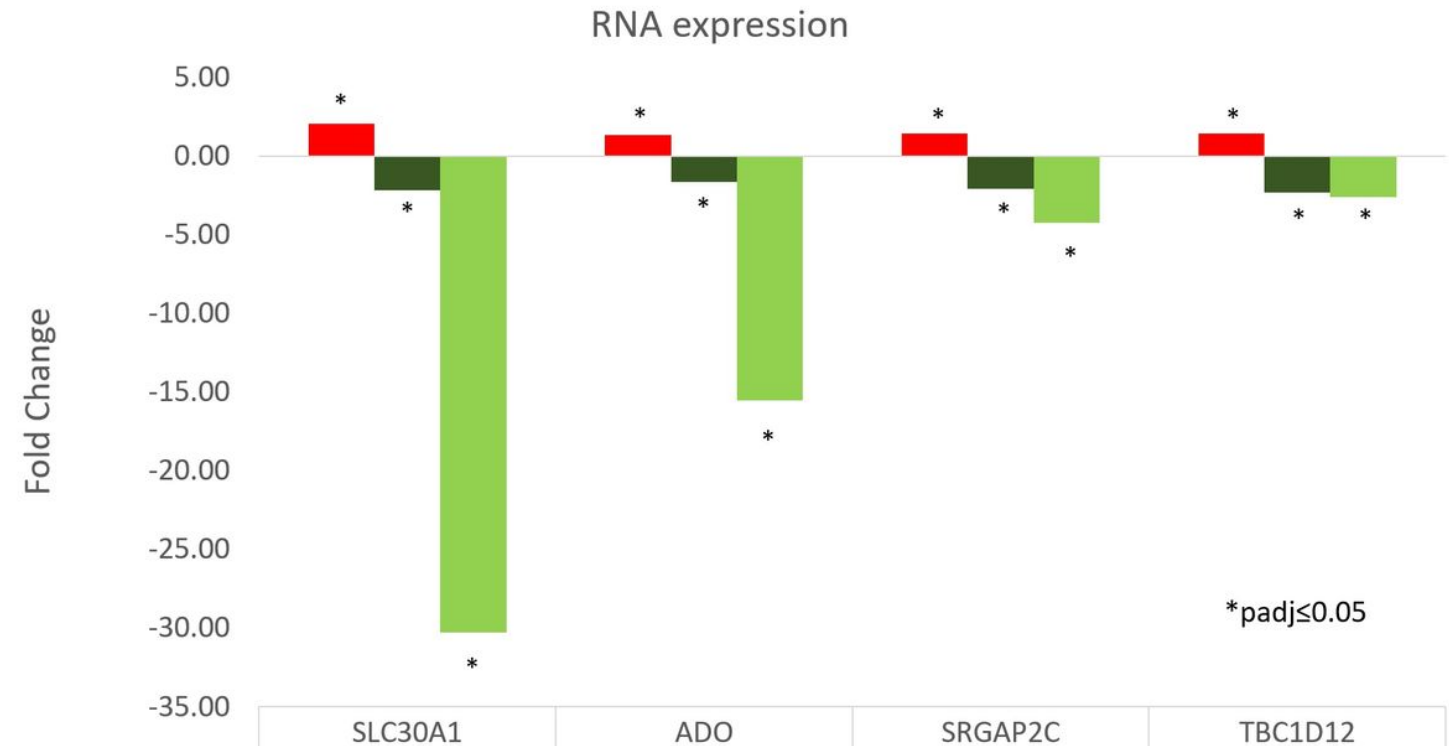
Figures



	SLC30A1	ADO	SRGAP2C	TBC1D12
PD vs CTRL	2.03	1.35	1.44	1.40
PCa vs CTRL	-2.18	-1.67	-2.06	-2.32
PCa_CTC vs CTRL	-30.27	-15.51	-4.24	-2.61

Figure 1

Histograms showing the distribution of fold change in PD patients compared to controls (PD vs CTRL), PCa patients compared to controls (PCa vs CTRL) and PCa CTC patients compared to controls (PCa_CTC vs CTRL).

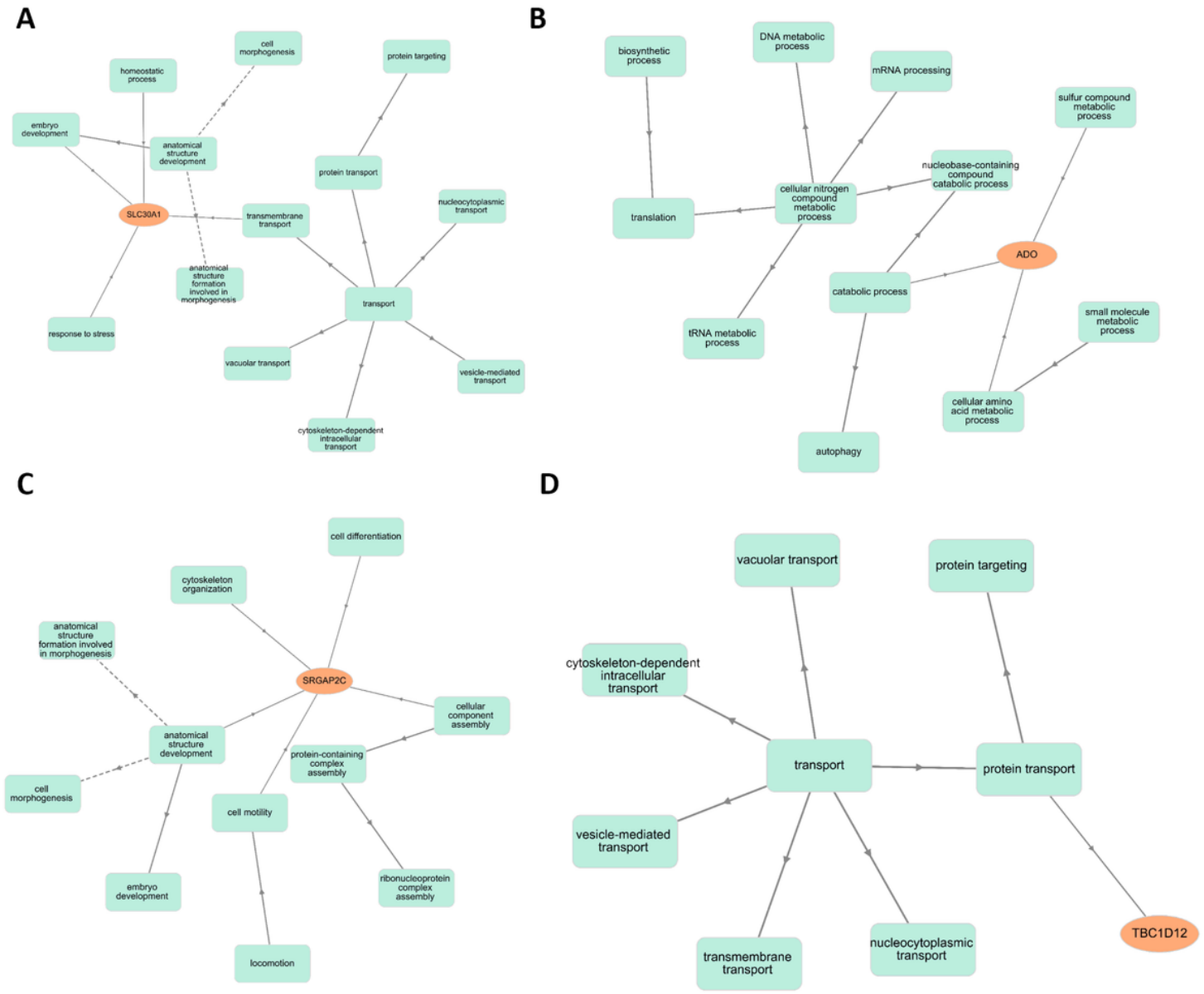


Figure 2

Gene Ontology annotation analysis of the differentially expressed transcripts in PCa and PD patients compared to healthy controls. Biological Process network of SLC30A1 (A), ADO (B), SRGAP2C (C) and TBC1D12 (D) genes.