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Review

POSSIBLE RELATED FUNCTIONS OF THE NON-HOMOLOGOUS CO-REGULATED GENE PAIR PDCD10 AND SERPINI1

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ABSTRACT

Gene expression in mammalians is a very finely controlled mechanism, and bidirectional promoters can be considered one of the most compelling examples of the accuracy of genic expression coordination. As recently reported, a bidirectional promoter regulates the expression of the PDCD10(whose mutations cause familial Cerebral Cavernous Malformations (CCMs) and SERPINI1 gene pair, even though they are non-homologous genes. The aim of this study was to identify any potential common roles of these two co-regulated genes. An *in-silico* approach was used to identify functional correlations, using the BioGraph, IPA® and Cytoscape tools and the KEGG pathway database. The results obtained show that PDCD10 and SERPINI1 may co-regulate some cellular processes, particularly those related to focal adhesion maintenance. All common pathways identified for PDCD10 and SERPINI1 are closely associated with the pathogenic characteristics of CCMs; we thus hypothesize that genes involved in these networks may contribute to the development of CCMs.

1. Introduction

Gene expression regulation is a very complex and intricately controlled mechanism. In higher eukaryotes, bidirectional promoters can be considered one of the most compelling examples of the accuracy of genic expressioncoordination. Most genepairs regulated by a common genomic region seem to have related functions, and over 10% of mammalian genes have been found to be regulated by a bidirectional promoter. This co-regulation mechanism is generally characteristic of genes encoding for proteins involved in the same cellular processes (e.g. chromatin remodeling, differentiation, proliferation, cell cycle regulation) [1]. However, microarray expression has analyses have shown that in a smaller fraction of genes, the existence of a bidirectional promoter determines a differential expression for the two genes (e.g. mouse genes TK/KF), as well as a mechanism of mutually exclusive expression [2]. In agreement with the hypothesis that this occurrence is indicative of a functional correlation forco-regulated genes, the gene pairs detected in the human genome were also observed in mice, and they were also found to be conserved during evolution [3]. Sequencing analyses of bidirectional promoters has allowed the definition of specific structural characteristics that distinguish these promoters from the traditional monodirectional ones: i) a shorter length (under 1000bp); ii) a higher GC-content (about 66% compared with the 53% of monodirectional promoters); iii) a lack of TATA boxes, regions, whereas CAAT boxes are common [1].Nevertheless, genes regulated by TATA-less unidirectional promoters have been found in mammals, such as the gene for the chemokine receptor CCR5, which regulates the trafficking and effector functions of memory/effector T-lymphocytes, macrophages, and immature dendritic cells. It also acts as the main co-receptor allowing the entry of R5 strains of human immunodeficiency virus[4].While the importance of co-regulation for two homologous genes is evident, it is more difficult to rationalize the existence of a bidirectional promoter for two genes that seemingly have no related functions. The two genes we describe in the present article, PDCD10 and SERPINI1, are non-homologous and appear to have no functional correlation; however, we think that they may contribute to the regulation of the same cellular processes through different signaling cascades, despite their expression patterns not being comparable.PDCD10is ubiquitously expressed, and has previously been identified as a positive regulator of apoptosis[5], whereas SERPINI1

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encodes for neuroserpin, a serine protease inhibitor that regulates tissue-type plasminogen activator (tPA); in adults, its expression is limited to few cerebral areas, including the hippocampus, amygdala and hypothalamus [6]. While PDCD10 mutations are a proven cause of Cerebral Cavernous Malformations (CCMs)[7],mutations of SERPINI1result inthe emergence of a defective protein that polymerizes and precipitates in neurons, and has been shown to contribute to the development of a neurodegenerative disease known asFENIB (Familial Encephalopathy with Neuroserpin Inclusion Bodies) [8] or bipolar disorder[9].These genes map on 3q26.1,with theSERPINI1 organized in a cluster with the homologous SERPINI2; PDCD10 and WDR49 are also located within the same cluster. SERPINI2 encodes for another inhibitor of tPAs[10]. The functions of WDR49remain unclear for the moment. The lack of extensive knowledge on these genes makes it more difficult to deduce any possible correlations. The asymmetric bidirectional promoter we investigated is an 851 bp TATA-less intergenic region that controls SERPINI1 in forward and PDCD10 in reverse, with a head-to-head configuration, a GC-content of about 65%, and two different CAAT boxes (GG T/C CAATCT) at a distance of about 100bp from each other. A 175 bp fragment (from nucleotides 1 to 175 in forward) functions as a minimal bidirectional promoter, and its deletion causes a total absence of transcriptional activity [11]. A non-canonical E-box (5'-CATGCG-3, nt 154-159) has been detected within this region, and found to act as a target site for c-Myc that trans-activates bidirectional transcription [12]. Minimal bidirectional promoter is included in a conserved 400 bp region that is presumed to contain enhancers and silencers for a differential expression of the two co-regulated genes. As discussed above, SERPINI1 has a homologous gene, SERPINI2, that maps at the same locus. Therefore, the existence of a bidirectional promoter for these two genes could be expected; however, it does not. In this article, we report the findings of an in-silico study on the PDCD10-SERPINI1 gene pair.

2. Methods

Bioinformatic resources offer a valuable support for genomic analytics. For the present study, an in-silico approach was used to investigate both the known and the inferred related functions of SERPINI1 and PDCD10. The commonly acknowledged related functions have been reported in previous studies, whereas the inferred ones derive from observations in other species, homologous genes, or other genes with related functions. The in-silico analysis was performed in three different phases: i) identification of pathways common for both genes; ii) collection of genes involved in common pathways; iii) intersections of these genes with PDCD10 and SERPINI1.

Identification of common pathways: The first phase of the analysis was the identification of pathways with involvement by each of the genes, in order to detect possible common pathways and, consequently, related functions. This phase was carried out using three different tools: Bio Graph (www.biograph.be), Ingenuity Pathway Analysis (IPA®, Quiagen), and Gene Ontology(GO) (www.geneontology.org). The Bi oGraph tool enables the detection of functions for single entities, like genes or diseases, or possible associations between two such entities; thus,"PDCD10" and "SERPINI1" were used as inputs in the "find links" section. The two genes were then individually considered for the IPA® analysis, using the "canonical pathway" filter. This tool detects a complete network of all the pathways in which each input gene is involved. The GO database provides an updated source of annotations regarding the molecular functions, cellular components and biological processes of most genes. The data available for PDCD10 and SERPINI1 were compared, and common pathways were evaluated.

Collection of genes involved in common pathways: common pathways were determined as described above, and the genes involved were obtained from the KEGG pathway database (www.kegg.jp), setting "Homo Sapiens" as the organism, and using the following keywords individually: "angiogenesis", "apoptosis", "tight junction", "adherens junction", and "focal adhesion".

PDCD10–SERPINI1network: The aim of this study was to establish molecular networks to explain how PDCD10 and SERPINI1 might be involved in each investigated pathway. These networks were generated with the Cytoscape software through the identification of intersections of genes selected from the KEGG database and PDCD10/SERPINI1. All common pathways were included and five intersections were generated. A search for associated phenotypes of genes related to PDCD10 and SERPINI1 was carried out in the Mala Cards Human Disease Database (www.malacards.org).

3. Results

Identification of common pathways

Analysis performed with the BioGraph tools howed that the two genes regulate the maintenance of focal adhesions, but they act through different pathways: PDCD10through thePP2A phosphatase cascade,andSERPINI1through the regulation of extracellular matrix (ECM) proteins mediated by tPA and plasmin (http://www.biograph.be/concept/graph/C1418402/C1418546).For the GO analysis, annotations regarding "biological processes" were considered, and the only common process identified was the regulation of apoptosis (table1). IPA® analysis revealed a more complex scenario,identifying271 and 51 pathways, for PDCD10 and SERPINI1respectively, 34 of which were common for the two genes. Many of these involved the biological processes involved in inflammatory response, particularly interleukin signaling; however, only those pertinent to angiogenesis, apoptosis, survival, tight and adherens junctions, and focal adhesions were included in the present study.

Collection of genes involved in common pathways

The precise functions of PDCD10 and SERPINI1are only partially known, and not included in the KEGG database. Therefore, for each previously selected cellular process, different KEGG pathways were considered; the complete list is shown in table 2. A total of 212, 750, 249, 636 and 565 genes were collected for angiogenesis, apoptosis, tight junction, adherens junction and focal adhesion processes, respectively; a full list of KEGG genes for each process is available upon request.

PDCD10–SERPINI1 network

Molecular network reconstruction performed with the Cytoscape tool allowed the identification of several points of convergence between the two non-homologous genes:PDCD10 and SERPINI1. In a network-style representation, each gene is considered a "node" while the connections are

named “edges”. At the first intersection, no node directly linked to both PDCD10 and SERPINI1 (primary nodes). Common nodes were obtained from intersections of the primary nodes, selected and extracted from global networks. For each cellular process considered, at least one gene was identified as a point of convergence between PDCD10 and SERPINI1(Figs.1 a-e).For angiogenesis, two related genes were found: MYC, that co-localizes with SERPINI1 and PDCD10; and CUL2, that is co-expressed with them. While MYC is known to regulate bidirectional promoter activity, CUL2 encodes for a protein involved in multiple cullin-RING-based ECS (ElonginB/C-CUL2/5-SOCS-box protein) E3ubiquitin ligase complex formation. For apoptotic process, only the MYC gene was identified as a common node. EPB41L3, encoding for a cytoskeleton-associated protein and previously recognized as a promoter of apoptosis, was found to be the point of convergence for the tight junction regulation process.

The networks for adherens junction and focal adhesion regulation were more complex. For the former, co-expressions ofSMAD2 and FZD6with the MYC gene were observed. SMAD2 is a modulator of TGF-β signaling, involved in multiple cellular processes, such as proliferation, apoptosis and differentiation. FZD6, instead, is a member of the 'frizzled' gene family, and encodes for a Wnt receptor, functioning as a negative regulator of the canonical Wnt/beta-catenin signaling cascade. For focal adhesion regulation, further points of convergence includedDAAM1 and ACTR3. DAAM1 is a regulator of cytoskeletal architecture; it contains two formin homology (FH) domains, and is implicated in the Wnt signaling-mediated maintenance of cell polarity. The precise functions ACTR3have not yet been determined; however ,it is known to take part in the formation of the ARP2/3 complex, involved in lamellipodia formation. For each of these genes, associated phenotypes were collected from the Mala Cards database. Briefly, common phenotypes were found to be mainly associated with leukemia, brain tumors and neurodegenerative diseases (not shown);full results are available upon request.

Finally, a network with theintersections of all common nodes with the three CCM genes and SERPINI1was generated(Fig.2). Two new important connotations were identified for SERPINI1: co-expression and co-localization with TRIM2, a gene that is mutated in arteriovenous malformations; and a physical interaction with DEDD, an activator of CASP3, like PDCD10.

Gene Symbol	GO Identifier	GO TermName	Evidence	Reference
PDCD10	GO:0001525	angiogenesis	IEA	UniProt Keywords2GO (UniProtKB/Swiss-Prot entries)
PDCD10	**GO:0006915**	**Apoptotic process**	**IEA**	**UniProt Keywords2GO (UniProtKB/Swiss-Prot entries)**
PDCD10	GO:0008284	positive regulation of cell proliferation	IDA	PMID:17360971
PDCD10	GO:0043066	negative regulation of apoptotic process	IDA	PMID:17360971
PDCD10	GO:0043406	positive regulation of MAP kinase activity	IDA	PMID:17360971
SERPINI1	GO:0007417	centralnervoussystemdevelopment	TAS	PMID:9070919
SERPINI1	GO:0007422	peripheralnervoussystemdevelopment	TAS	PMID:9070919
SERPINI1	**GO:0008219**	**celldeath**	**IEA**	**UniProt Keywords2GO (UniProtKB/Swiss-Prot entries)**
SERPINI1	GO:0010466	negative regulation of peptidase activity	IEA	UniProt Keywords2GO (UniProtKB/Swiss-Prot entries)
SERPINI1	GO:0010951	negative regulation of endopeptidase activity	IBA	GO_REF:0000033
SERPINI1	GO:0030155	regulation of celladhesion	IEA	Ensembl Compara
SERPINI1	GO:0030162	regulation of proteolysis	IBA	GO_REF:0000033

Table 1 - Gene Ontology annotations for “Biological process” for the PDCD10 and SERPINI1 genes.

KEGG PATHWAY			EXTRACTED GENES
ANGIOGENESIS	hsa04370	VEGF signalingpathway	212
	hsa04066	HIF-1 signalingpathway	
	hsa04350	TGF-beta signalingpathway	
APOPTOSIS	hsa04210	Apoptosis	750
	hsa04668	TNF signalingpathway	
	hsa04350	TGF-beta signalingpathway	
	hsa04151	PI3K-Akt signalingpathway	
	hsa04390	Hipposignalingpathway	
	hsa04064	NF-kappa B signaling pathway	
	hsa04722	Neurotrophinsignalingpathway	
TIGHT JUNCTIONS	hsa04530	Tight junction	249
	hsa04514	Cell adhesionmolecules (CAMs)	
ADHERENS JUNCTIONS	hsa04520	Adherens junction	636
	hsa04015	Rap1 signalingpathway	
	hsa04390	Hipposignalingpathway	
	hsa04514	Cell adhesionmolecules (CAMs)	
	hsa04810	Regulation of actincytoskeleton	
FOCAL ADHESIONS	hsa04510	Focal adhesion	565
	hsa04015	Rap1 signalingpathway	
	hsa04512	ECM-receptorinteraction	
	hsa04310	Wntsignalingpathway	
	hsa04810	Regulation of actincytoskeleton	

Table 2 - KEGG pathways for cellular processes common for the CCM3-SERPINI1 gene pair. Full list of extract genes is available upon request.

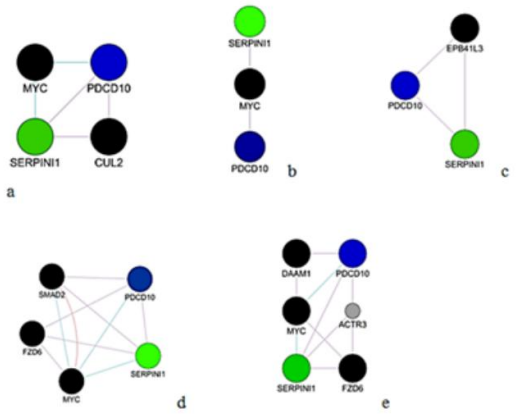

Figure 1 - Cytoscape tool results: common primary nodes for the PDCD10-SERPINI1 gene pair (blue and green nodes, respectively) obtained from intersections with genes extracted from the KEGG database, for each cellular process considered. (a) angiogenesis; (b) apoptosis; (c) tight junctions; (d) adherens junctions; (e) focal adhesions. Common nodes are marked in black.

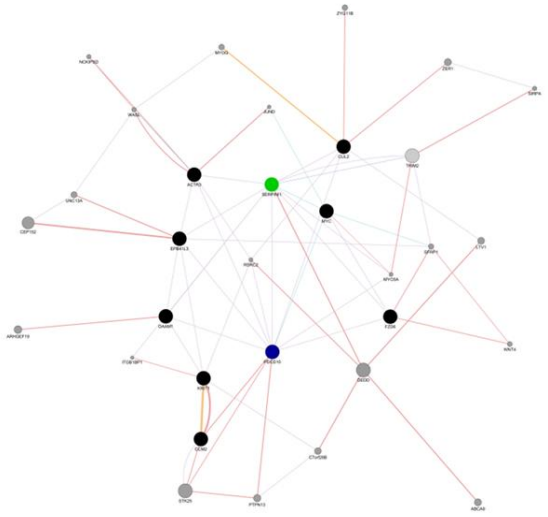

Figure 2 - Global network obtained with Cytoscape from intersections of common nodes for the PDCD10-SERPINI1 gene pair and KRIT1 and CCM2, the other two genes causing CCMs.

4. Discussion

As reported, both PDCD10 and neuroserpin can contribute to neurovascular system morphogenesis. Specifically, PDCD10 acts through apoptosis control, while neuroserpin acts through synapse promotion and, against tPA, through ECM remodeling. As shown by the pathway analysis, focal adhesion regulation seems to be the most important functional correlation. Particularly, Bio Graph data highlights how PDCD10 acts through an intracellular pathway associated with phosphatases of the STRIPAK complex, while SERPINI1 acts through extracellular signaling mediated by tPA and its effect son ECM proteins, especially laminins and fibronectin, that bind integrins and control the expression of genes involved in proliferation, apoptosis, motility and cellular adhesion. SERPINI1 was previously reported as a prognostic factor in colon cancer due to its role in cellular adhesion: a reduced SERPINI1 expression was found to cause adhesion loss[13]. Absence of SERPINI1 in cerebral, gastric and colorectal tumors caused by genic silencing was recently found to be associated with an increased loss of cellular adhesion [14]. Moreover, expression of both PDCD10 and SERPINI1 was observed in exosomes isolated from expressed prostatic secretions [15, 16]. PDCD10 co-localization with the GCKIII complex and GM130 protein in the Golgi apparatus was found to be essential for maintaining Golgi integrity [17]; failed cell polarization and decreased GCKIII kinase activity following a PDCD10 deficit was observed and found to be caused by kinase degradation by the ubiquitin-proteasome system. GCKIII is produced by the three proteins: Mst4, STK24 and STK25, which are involved in cell cycle regulation, maintenance of structural integrity of both the Golgi apparatus and cytoskeleton,and development of hyperglycemia and hyperinsulinemia [18]. Recent evidence suggests apleiotropic role for PDCD10, reflecting its ability to modulate oxidative stress response [19]. Furthermore, PDCD10 was identified as a key modulator of the VEGF pathway due to its VEGFR2-stabilizing action[20]. An interaction between PDCD10 and PCDHγ was reported to be involved in the physiological pro apoptotic signaling process in neuronal cells; similarly, down regulation of the apoptotic process was found to cause angiogenesis alterations, due to a PDCD10 deficit followed by a DLL4-Notch signal alteration [21].For CCMs, a very important observation regarding malformation localization had been made: germ-line mutation carriers develop lesions mainly at brain capillaries, despite the fact that the three genes are ubiquitously expressed from the embryonic stage. This clearly indicates that the unique microenvironment, that enables a correct blood-brain barrier (BBB) development, surely contributes to CCM pathogenesis, which hence cannot be considered solely due to an endothelial dysfunction; rather, CCMs result from cross-talk alterations among the different neurovascular unit(NVU) components.

The existence of communication mechanisms between vascular and cerebral tissues is already observable during embryonic development: appropriate vasculogenesis is essential for a correct nervous system formation. To reflect this interdependence, the expression "neurovascular unit" was coined. BBB dysfunctions often arise from alterations at tight and adherens junctions and focal adhesions, as well as out-and-out "communication deficits" among the different cell types. One of the characteristic features of CCMs is the lack of pericytes that secrete many growth factors, including TGF-β, essential for the angiogenetic process[22]. In this context, non-conventional nervous system-specific activity of coagulation proteases, including tPA, seems coherent [23]. TGF-β acts on astrocytes and reduces tPA expression in brain microvascular endothelial cells (BMECs), leading to an increased permeability by means of a c-AMP dependent signal transduction pathway, through protein kinase A and Epac that modulates VE-cadherin junction integrity and actin reorganization [24]. At the same time, a role was recently reported for tPA in the cytoskeletal modeling of astrocytes. This mechanism is based on the interactions of tPA and plasminogen (Plgn) with their receptors on the surface of astrocytes. The plasmin thus produced activates MAPK (involved in the stress-activated Mitogen-activated protein kinase signaling cascade) [25], PI3K, and Rho/ROCK pathways, resulting in cytoskeletal remodeling and increased BBB permeability in the astrocytes. In addition, it also plays a role as a cell adhesion modulator through the regulation of N-cadherin levels [26]. These evidence show how neuroserpin activity can control endothelial barrier integrity against tPA regulation, thus confirming the importance of maintaining a proper tPA-neuroserpin balance. Finally, the network created with the intersections of PDCD10, SERPINI1, their common nodes, KRIT1 and CCM2seems to be noteworthy. The co-localization and co-expression of SERPINI1andTRIM2 appears to be particularly significant. TRIM2is a gene encoding for a protein that belongs to the "tripartite motif" family. Its functions are still unknown; however, TRIM2 mutations have been linked to multiple sclerosis, neurodegenerative diseases and arteriovenous malformations (AVMs). AVMs are a type of benign cerebral vascular lesions characterized by the absence of capillaries, resulting in an arteriovenous continuity with mixing of arterial and venous blood. Moreover, the lack of capillaries causes a rapid hematic flow in the affected area, preventing an appropriate oxygenation of the cerebral parenchyma. These lesions seem to be linked to sporadic forms of CCM with multiple lesions. As per the Mala Cards database, this association seems to be mediated by the two genes PECAM1, encoding for membrane antigen CD31, and KDR, that produces the R2 isoform of the VEGF receptor. Unfortunately, the current limited understanding of the actions of SERPINI1 makes these associations difficult to explain. However, such relations can be considered as a potential investigative element in CCM patients affected by sporadic forms of the disease with multiple lesions, negative for the three currently known genes.

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5. Conclusions

According to our results, the PDCD10 and SERPINI1 genes may act on the same pathways but through different molecular patterns. The possibility that the two genes may somehow perform parallel functions becomes more plausible in view of the functions of SERPINI1, especially those related to cell junctions and synapse regulation. The aim of this article was to demonstrate that although these two genes are non-homologous, a functional relation between PDCD10 and SERPINI1 does exist, and it could imply the presence of a shared promoter, especially when the differential expression of the two co-regulated genesis taken into consideration. Particularly, nervous tissue tropism, both of neuroserpin and the other serine proteases, usually involved in the coagulation cascade, might contribute to CCM lesion development in brain capillaries. Moreover, the hypothesis that other genes involved in the pathways common to PDCD10 and SERPINI1 could contribute to CCM development was addressed. Within this context, we identified some genes that may provide a genetic framework for future genetic association studies.

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