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SHORT COMMUNICATION

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Alternative splicing of *G6PC2*, the gene coding for the islet-specific glucose-6-phosphatase catalytic subunit-related protein (IGRP), results in differential expression in human thymus and spleen compared with pancreas

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Abstract *Aims/hypothesis:* Autoimmunity to insulin, glutamic acid decarboxylase and the tyrosine-phosphatase-like protein IA-2 is associated with type 1 diabetes. The production of self-molecules in thymus and secondary lymphoid tissues is critical for self-tolerance; reduced levels may impair tolerance and predispose to autoimmunity, as shown for insulin. Alternative splicing causes differential expression of IA-2 gene (*PTPRN*) transcripts and IA-2 protein in human thymus and spleen compared with pancreas. IA-2 sequences not present in lymphoid tissues become autoimmune targets in type 1 diabetes. The beta cell molecule islet-specific glucose-6-phosphatase catalytic subunit-related protein (IGRP) is an autoantigen in the non-obese diabetic (NOD) mouse, a model of type 1 diabetes. IGRP is a candidate autoantigen in the human disease, but robust assays for IGRP autoantibodies and/or autoreactive T cells are not available. Both full-length and IGRP splice variants encoded by the *G6PC2* gene are expressed in the pancreas. In this study we tested the hypothesis that IGRP splice variants could be differentially expressed in thymus and spleen compared with the pancreas. *Methods:* We evaluated the expression of *G6PC2* transcripts in matched human thymus, spleen and pancreas specimens by RT-PCR. *Results:* Alternative splicing results in differential expression of *G6PC2* transcripts in thymus and spleen compared with pancreas. The full-length transcript is expressed in human pancreas but not in thymus or spleen. Five alternative spliced forms are always expressed in pancreas but those lacking exons 2, 3 and 4, alone or in combination, were rarely detected in thymus or spleen. *Conclusions/interpretation:* Differential tissue expression might favour autoimmune responses to IGRP in humans; target epitopes may be encoded by exons 3 and 4, or at the junctions of the conserved exons in the spliced transcripts. This information may aid in designing synthetic peptides for the identification of IGRP-specific autoreactive T cells in patients with type 1 diabetes.

Keywords Alternative splicing · *G6PC2* · IGRP · Thymus · Tolerance · Type 1 diabetes

Abbreviations IA-2: tyrosine-phosphatase-like protein · IGRP: islet-specific glucose-6-phosphatase catalytic subunit-related protein

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Introduction

Type 1 diabetes is an autoimmune disease resulting in the destruction of pancreatic beta cells and loss of insulin

secretory function. Autoimmune diabetes also develops in the non-obese diabetic (NOD) mouse. Many genetic and immunological features are common to mouse and man, including the specific targets of autoimmune responses (insulin/proinsulin, glutamic acid decarboxylase and a tyrosine phosphatase-like protein called IA-2).

The natural synthesis of insulin and other self-molecules in the thymus promotes self-tolerance (reviewed in [1]). Self-molecules, including insulin, are also produced in secondary lymphoid organs (spleen and lymph nodes). In both thymus and secondary lymphoid organs, self-molecules are produced via a transcription-dependent mechanism mediated by dendritic cells [2]; thymic epithelial cells have also been shown to transcribe genes coding for self-molecules [3]. Variation in the thymic synthesis of self-molecules may result in suboptimal tolerance and allow a greater proportion of potentially autoreactive T cells to escape into the periphery, where they can be triggered and cause autoimmunity. For example, levels of insulin gene transcription in the thymus are controlled by alleles at the *IDDM2* susceptibility locus. Higher levels of thymic transcription are associated with diabetes-protective *IDDM2* alleles and confer protection from diabetes [4]. In the NOD mouse, lack of expression of the insulin II gene (*Ins2*) in the thymus results in heightened diabetes incidence and severity, whereas *Ins2* overexpression in the thymus protects against diabetes (reviewed in [1]).

Another mechanism regulating the synthesis of self-molecules in the thymus is alternative splicing. This has been shown to cause differential expression of the gene encoding IA-2 (*PTPRN*) and lack of full-length protein in human thymus and spleen compared with the pancreas [5]. IA-2 sequences that are not expressed in the thymus and spleen are known targets of autoimmune responses in type 1 diabetes. Similarly, a proteolipid protein epitope that is not produced in the thymus because of alternative splicing is an autoantigen in experimental autoimmune encephalomyelitis, a model of multiple sclerosis [6].

Islet-specific glucose-6-phosphatase catalytic-subunit related protein (IGRP) is a 355 amino acid glycoprotein embedded in the endoplasmic reticulum and is selectively produced in pancreatic beta cells (reviewed in [7]). IGRP is an autoantigen in the NOD mouse, in which CD8 and CD4 T cell epitopes have been defined [8, 9]. In man, the gene encoding IGRP, *G6PC2*, lies next to a susceptibility locus on chromosome 2q31 [7], *IDDM7*, suggesting that IGRP may be an autoantigen in humans as well. However, there is presently no published evidence that autoimmune responses to IGRP occur in patients with type 1 diabetes, perhaps because of the lack of robust assays to detect humoral and/or cellular responses to this molecule. *G6PC2* contains five exons; both full-length and alternatively spliced transcripts have been reported in the pancreas [10]. We investigated whether, as is true for IA-2 and proteolipid protein, alternative splicing results in differential expression of *G6PC2* transcripts in thymus and spleen compared with the pancreas. If such a pattern were found, it would support the likelihood that IGRP is an autoantigen in human type 1 diabetes and might help to pinpoint regions of the protein that might be more likely to contain autoantigenic epitopes.

Materials and methods

Tissues

We studied frozen specimens of pancreas, thymus and spleen from fetal and neonatal autopsies obtained from the University of Miami Brain and Tissue Bank for Developmental Disorders. We could obtain 13 cases for which we had thymus, spleen and pancreas available. Estimated gestational age ranged between 22 and 40 weeks; one case was a 45-day-old infant. Cases included four males and six females; sex was unknown for three cases. Race was known for ten of the 13 cases, which included four individuals of European origin, three Hispanics and three African–Americans. Tissues were obtained with the approval of the Institutional Review Board of the University of Miami.

Analysis of IGRP mRNA expression by RT-PCR

Thymus, spleen and pancreas RNA was extracted using the Micro-to-Midi Total RNA Purification System (Invitrogen, Carlsbad, CA, USA) using 100 mg of tissue. Total RNA was treated with DNase (Deoxyribonuclease I, Amplification Grade; Invitrogen) to remove any contamination with genomic DNA. cDNA synthesis (by RT) was performed using the SuperScript First-Strand Synthesis System and random hexamers. cDNA samples were amplified by PCR using SuperTaq Plus Polymerase (Ambion, Austin, TX, USA). All reactions were run for 40 cycles; annealing and extension temperatures were 55 and 68°C, respectively. The sequences of the IGRP primers were: forward, 5′-CCAAGATGATATGGGTAGC-3′; reverse, 5′-TGTCAA TGTGGATCCAGTC-3′. They are almost identical and produce the same amplification pattern as that reported previously [10]: the primers amplify a full-length transcript of 594 bp encompassing nucleotides 164–757 (exons 1–5). In addition, these primers can detect the alternative five spliced forms reported in pancreas lacking exon 4, exons 3 and 4, exons 2 and 3, exons 2 and 4, and exons 2, 3 and 4, which were verified by sequencing [10]. Because the various transcripts were amplified in the same reaction with the same primers, the intensity of the bands reflects the relative abundance of each transcript in each sample. We performed control RT-PCR reactions for *GADPH*, a housekeeping gene. Primers for *GADPH* were: forward,

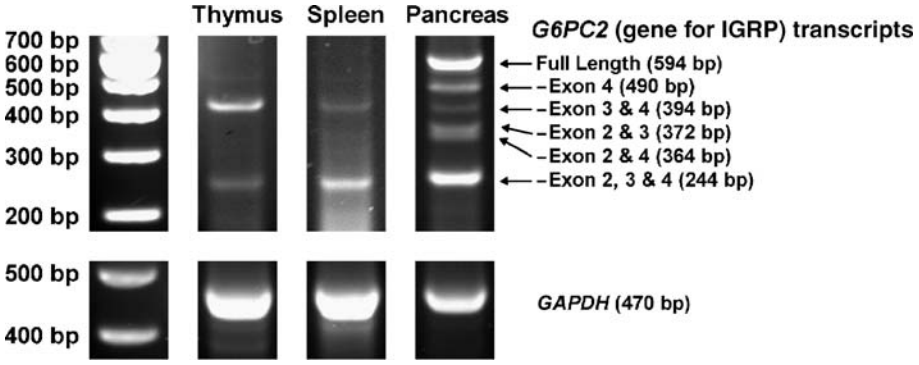

Fig. 1 Evaluation of *G6PC2* mRNA expression by RT-PCR. Representative example illustrating the pattern of expression of the *G6PC2* transcripts observed in pancreas compared with thymus and spleen. Molecular weight markers and control lanes for *GAPDH* are shown

5′-CCATGGAGAAGGCTGGGGGCTC-3′; reverse, 5′-CCTTCTTGATGTCATCATATTTGGCA-3′. Negative controls included reactions lacking template and reactions in which the reverse transcriptase had been omitted. RT-PCR products were electrophoresed on 2.2% agarose gels

Table 1 *G6PC2* mRNA expression in thymus, spleen and pancreas in the individuals studied

Case	Full length (594 bp)	Exon 4 (490 bp)	Exons 3 and 4 (394 bp)	Exons 2 and 3 (372 bp)	Exons 2 and 4 (368 bp)	Exons 2, 3 and 4 (244 bp)
3687						
Thymus	−	−	+	−	+	+
Spleen	−	−	+	+	+	+
Pancreas	+	+	+	+	+	+
3708						
Thymus	−	−	−	−	+	+
Spleen	+	+	+	+	+	+
Pancreas	+	+	+	+	+	+
3754						
Thymus	−	−	+	−	+	+
Spleen	−	−	−	+	+	+
Pancreas	+	+	+	+	+	+
3771						
Thymus	−	−	−	+	+	−
Spleen	−	−	−	+	+	−
Pancreas	+	+	+	+	+	+
3815						
Thymus	+	−	−	−	+	+
Spleen	−	−	−	+	+	+
Pancreas	+	+	+	+	+	+
3827						
Thymus	−	−	−	−	+	−
Spleen	−	−	−	−	+	−
Pancreas	+	+	+	+	+	+
1264						
Thymus	−	+	+	−	+	+
Spleen	−	−	+	−	+	+
Pancreas	+	+	+	+	+	+
3658						
Thymus	−	−	−	−	+	+
Spleen	−	−	−	−	+	+
Pancreas	+	+	+	+	+	+
3794						
Thymus	−	−	−	+	+	−
Spleen	−	−	−	+	+	−
Pancreas	+	+	+	+	+	+

and identified based on published fragment size using a molecular weight marker (100 bp ladder).

Results

We compared the expression of *G6PC2* transcripts in the thymus, spleen and pancreas obtained from 13 autopsy cases. Studying tissues from the same individuals allowed the assessment of tissue-specific splicing independently of individual variation. Because mRNA degradation can be a significant problem with human autopsy tissues, we considered for analysis those cases in which *GADPH* transcripts were present in all three tissues and in which at least the smallest *G6PC2* splice variant was detected in thymus, spleen and pancreas (all transcripts examined were amplified in the same reaction by the same primers). Nine of 13 cases were suitable for analysis according to these criteria. A representative example of the splice variants analysed by RT-PCR is shown in Fig. 1. Essentially all pancreas samples expressed the full-length transcript, as well as the splice variants lacking exon 4, exons 3 and 4, exons 2 and 3, exons 2 and 4, or exons 2, 3 and 4 (Tables 1 and 2). None (0/9) of the thymus and 1/9 spleen samples expressed the full-length transcript. None (0/9) of the thymus and 3/9 spleen samples expressed the alternative spliced transcript lacking exon 4. The spliced variant lacking both exons 3 and 4 was found in 2/9 and 3/9 thymus and spleen specimens, respectively. The variants lacking exons 2 and 3 or exons 2 and 4 were detected in 0/9 and 2/9 thymus specimens, and 5/9 and 3/9 spleen specimens, respectively. The smallest spliced variant lacking exons 2, 3 and 4 was the one most frequently detected in thymus and spleen specimens (7/9). Thus, the full-length transcript and the splice variant lacking exon 4 were consistently absent in the thymus, or expressed at much lower levels than other variants, below the detection limit of our assay.

Discussion

The natural, 'ectopic' production of insulin and other self-molecules in the thymus and secondary lymphoid organs is believed to promote self-tolerance (reviewed in [1]). Mechanisms that quantitatively and qualitatively influence the production of self-molecules in lymphoid tissues may negatively affect tolerance and may represent a permissive factor for the development of autoimmunity. Alternative splicing may influence gene transcription and protein synthesis, so that the immune system may not be tolerant to epitopes that are not available for presentation in the thymus and spleen, or that are only present in the peripheral organ that primarily produces that protein. For example, epitopes targeted by autoimmune responses to IA-2 and proteolipid protein have been mapped to portions of the molecules that are not produced in the thymus. In both cases, the exon encoding autoreactive epitopes is not expressed in the thymus because of alternative splicing, while the target organ expresses the full-length protein [5, 6]. In addition, the pancreas expresses a splice variant of IA-2 that is not observed in the thymus and spleen [5].

We show that alternative splicing results in differential expression of *G6PC2* transcripts in the thymus and spleen compared with the pancreas. Five major transcripts were always expressed in the pancreas. While it is not clear that all alternative spliced transcripts can be translated into protein, our key observation is that the full-length transcript was essentially not detected in thymus and spleen. Similarly, the splice variant lacking exon 4 was almost never detected in thymus. We suggest that the lack of expression or severely reduced expression of the full-length IGRP in thymus and spleen may lead to incomplete tolerance to this molecule, and support the hypothesis that IGRP might be an autoantigen in human type 1 diabetes. Our findings also suggest that autoreactive epitopes may be primarily encoded by exons 3 and 4. The exon junctions in the alternatively spliced forms (exons 2–5 and 3–5) may create sequences that are not present in the thymus and spleen and may be more likely to become target epitopes, depending in part on their binding affinities to different HLA molecules. This information may aid in the design of synthetic peptides for the identification of IGRP-specific autoreactive T cells in patients with type 1 diabetes, an effort currently ongoing in several research laboratories.

Table 2 Overall frequency of *G6PC2* transcripts in thymus, spleen and pancreas

	Thymus	Spleen	Pancreas
Full-length	0/9	1/9	9/9
Exon 4	1/9	3/9	9/9
Exons 3 and 4	2/9	3/9	9/9
Exons 2 and 3	0/9	5/9	9/9
Exons 2 and 4	2/9	3/9	8/9
Exons 2, 3 and 4	7/9	7/9	9/9

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