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Indoleamine 2,3-dioxygenase inhibition alters the non-coding RNA transcriptome following renal ischemia-reperfusion injury

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ABSTRACT

Background: Indoleamine 2,3 dioxygenase (IDO) degrades the essential amino acid tryptophan and has been shown to minimize rejection in animal models of renal transplantation. Ischemia-reperfusion injury (IRI) is unavoidable in renal transplantation and correlates with shorter graft survival times. Despite its favorable effects on rejection, there is evidence that IDO may facilitate renal IRI. Differentiating the negative impact of IDO on IRI from its pro-tolerant effects in allograft rejection is of clinical relevance. In these studies we hypothesized that constitutive IDO activity may influence renal genes associated with recovery from IRI, and that IDO inhibition may unmask these effects.
Methods: We examined the renal transcriptome in a rat model of IRI with and without IDO inhibition with 1-methyl-D-tryptophan (1-MT), and assessed for alterations in the gene expression signature.
Results: These studies demonstrated that during recovery from renal IRI, pre-treatment with 1-MT alleviated alterations in 105 coding sequences associated with IRI, and in turn triggered new changes in 66 non-coding transcripts, the majority of which were represented by small nucleolar RNA.
Conclusion: These results suggest a biologic role for non-coding, IDO-dependent genes in regulating the early response to IRI.

1. Introduction

Indoleamine 2,3 dioxygenase (IDO) is a heme containing enzyme degrading the essential amino acid tryptophan [1]. IDO-mediated tryptophan depletion and/or the accumulation of degradation products have been shown to facilitate immune regulation through the induction of regulatory T cell expression [1]. In this regard, IDO enzymatic activity has been shown to mediate maternal–fetal tolerance [2] and reduce rates of rejection in rodent models of kidney transplantation [3].

All renal transplants are associated with ischemia-reperfusion injury (IRI). IRI correlates with the development of chronic allograft nephropathy and shorter graft survival times [4]. Despite its pro-tolerant properties, IDO has been implicated in facilitating IRI in the kidney. In this regard, Mohib et al. [5] showed that IDO was rapidly upregulated in the kidneys of mice following IRI, with prominent positive immunohistochemical staining observed in tubular epithelia, and that inhibition of IDO with 1-methyl-D-tryptophan (1-MT) or knockout of IDO was protective against renal IRI. The same group previously demonstrated that IDO upregulation in renal tubular cells increased apoptotic cell death [6]. These data indicate that IDO may have dual clinical effects in renal transplantation — the facilitation of immunologic tolerance, and at the same time enhanced tubular susceptibility to injury. Separating the negative impact of IDO on IRI from its pro-tolerant effects on rejection is relevant to defining any potentially therapeutic role for the enzyme in solid organ transplantation. In this regard, unique changes in gene expression patterns from kidneys exposed to IRI and treated with an IDO blocker may give clues to the molecular mechanisms underpinning the role of IDO in the early response to IRI.

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2. Objective

In these studies we hypothesized that IDO activity in the peri-ischemic period may influence the kidney transcriptome associated with the response to renal IRI. To address this question, we utilized a rat model of renal IRI with and without IDO inhibition using 1-MT, and assessed alterations in the gene expression signature from the renal cortex. The results of these studies demonstrated that during the early reperfusion phase, pre-treatment with 1-MT alleviated changes in 105 coding sequences associated with renal ischemia, and in turn triggered new alterations in 66 non-coding transcripts. These results suggest a biologic role for non-coding, IDO-dependent genes in regulating the early response to IRI.

3. Material and methods

3.1. Experimental design

All experiments were conducted in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* and approved and monitored by the Georgia Regents University Institutional Animal Care and Use Committee. Studies were performed on four groups of male Sprague–Dawley rats (N = 5 rats/group, 10–12 wks of age; Jackson Laboratories, Bar Harbor, ME). IDO inhibition was attained by pretreating rats with the IDO inhibitor 1-methyl-D-tryptophan (1-MT) (Sigma, St. Louis, MO), 140 mg/kg po 1 and 24 h prior to clamping the renal arteries. Separate groups of rats underwent IRI or Sham surgery, with or without pretreatment with 1-MT. Following IRI or Sham surgery, the total RNA was extracted from the left renal cortex and hybridized to a rat gene microarray (GeneChip®Rat Gene 1.0 ST Array, Affymetrix) (see below).

3.2. Renal ischemia-reperfusion injury

Each rat was anesthetized with thiobutabarbital sodium (100 mg/kg ip; Sigma Chemical Co., St Louis, MO), placed on a servo-controlled heating table in the supine position, and maintained at 37 °C throughout the experiment. The kidneys were approached via anterior abdominal incision followed by the placement of small microvascular clamps on the renal arteries, stopping blood flow. After a period of 30 min, the clamps were removed, allowing reperfusion. Following 60 min of reperfusion, the animals were sacrificed and the left kidney taken for study. Sham animals underwent the same procedure without renal artery clamping.

3.3. Determination of the renal transcriptome

All kidneys were snap-frozen in liquid nitrogen. Frozen cortical tissue samples (30 mg) were ground into a fine powder in a mortar cooled by dry ice. Total RNA was isolated using Ambion PureLink RNA mini kit (Life Technologies, Carlsbad, CA), the quality of each sample was analyzed on a 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA) and assured by a RNA Integrity Number ≥7. Ambion WT expression kit (Life Technologies, Carlsbad, CA) was used to generate sense strand cDNAs from 250 ng of the total RNA. The sense strand cDNAs (5.5 μg) were fragmented, biotin labeled and hybridized to the GeneChip®Rat Gene 1.0 ST arrays (Affymetrix). After 16 h of hybridization, the arrays were washed, stained and scanned (Affymetrix GeneChip Scanner 3000). Expression data were obtained in the form of .CEL files.

3.4. Analysis of gene expression

Analysis of gene expression was performed on the .CEL files from above. Quality of the arrays was assessed using probe level models and no arrays appeared to be anomalous. Analyses were conducted using transcript cluster probe sets. Probe signals were quantile-normalized and RMA was used for background subtraction. Only probe sets having at least an interquartile range of 0.25 were used for all subsequent analyses. All processing used the oligo package in R. One array per rat was used, resulting in 20 arrays (five arrays in each of the 4 groups). The package LIMMA (Smyth GK, Limma: linear models for microarray data. In Bioinformatics and Computational Biology solutions using R and Bioconductor, 397–420, 2005) was used to test for differential expression. We used pairwise comparisons in LIMMA to examine the differences between groups and used the false discovery rate to adjust for multiple testing. Annotations were provided by Affymetrix, using transcript cluster annotations (release 32). RNA category enrichment was assessed using Fisher's exact test. The Database for Annotation, Visualization and Integrated Discovery (DAVID) v6.7 was used for functional annotation clustering [7].

4. Results

4.1. Gene expression data

The array contains over 27,000 gene-level probe sets, of which 8711 exhibited an interquartile range of 0.25, and were thus included in the. We termed all probe sets that targeted previously known genes as "coding mRNA". Any probe set that targeted presumptive genes was termed cDNA or cDNA pseudogene. Non-coding RNA was categorized as transcriptional RNAs (ribosomal RNA (rRNA) and transfer RNA (tRNA)), microRNA (miRNA), small nuclear RNA (snRNA), and small nucleolar RNA (snoRNA) [8]. Additional transcripts present on the chip included miscellaneous RNA, mRNA from protein, TCR α joining region, and LINE sequences.

4.2. Pairwise comparisons

The pairwise comparisons performed in these studies are expressed as four comparisons. Comparison 1 assessed the effect of IRI on a VEH-associated transcriptome, and served as a positive control for the effects of IRI. Comparison 2 assessed the effect of IRI on a 1-MT-associated transcriptome and served as a positive control for 1-MT in IRI. Comparison 3 was the primary focus of these studies and examined the effect of 1-MT on an IRI-associated transcriptome and tested the effect of 1-MT in IRI. Comparison 4 investigated the effect of 1-MT on the Sham operated-associated transcriptome and acted as a positive control for 1-MT in Sham operated rats.

The transcripts significantly altered in each pairwise comparison are summarized in the following:

Comparison 1 — IRI vs Sham with vehicle. 108 transcripts were significantly altered and represented by four groups: cDNA (N = 3), mRNA (N = 102), tRNA (N = 1), and snoRNA (N = 2). 27% (28/102) of coding mRNA sequences had an adjusted p value of <0.001 (Supplemental materials).

Using DAVID, the most common functional categories of the 105 significantly altered coding transcripts from this comparison included apoptosis, biosynthetic processes, RNA-associated processes, and transcription-associated processes, with 17–29 transcripts represented in each category. These results are summarized in Table 1.

Comparison 2 — IRI vs Sham with 1-MT. 735 transcripts were significantly altered and represented by eight groups: cDNA (N = 40), cDNA pseudogene (N = 8), mRNA (N = 665), tRNA (N = 2), miRNA (N = 1), snRNA (N = 8), snoRNA (N = 10) and miscRNA (N = 1).

Comparison 3 — 1-MT vs vehicle in IRI. 84 transcripts were significantly altered and represented by six groups: cDNA (N = 12), mRNA (N = 6), rRNA (N = 13), tRNA (N = 1), snRNA (N = 2) and snoRNA (N = 50). There were 18 significantly altered coding sequences (Table 1). The most common transcripts altered were non-coding genes and included rRNA (N = 13) and snoRNA (N = 50).

The snoRNA sequences represented the majority of the non-coding transcripts. These genes were classified as cajal body (N = 3), box H/ACA (35), box CD (4) or otherwise unclassified (8). Table 3 lists each transcript according to published nomenclature. A complete list of the 66 non-coding RNAs significantly altered in this comparison is shown in the supplemental materials.

Comparison 4 —- 1-MT vs vehicle in Sham. No transcripts were significantly altered.

Significantly altered sequences (both coding and non-coding) shared between Comparisons 1, 2 and 3. Fig. 1A summarizes the shared significantly altered coding sequences between Comparisons 1, 2 and 3. Comparisons 3 and 2 both altered a single gene, HF-1 (hypoxia-inducible factor 1, RNA accession: NM_016993). Comparisons 2 and 1 shared 104 common genes. There were no common coding sequences between Comparisons 3 and 1.

Fig. 1B summarizes the shared significant non-coding sequences between Comparisons 1, 2 and 3. Comparisons 3 and 2 exhibited no overlap, nor did Comparisons 3 and 1. There were three common non-coding sequences between Comparisons 2 and 1, a single t-RNA and 2 snoRNAs.