BBA Clinical 5 (2016) 66–71

Contents lists available at ScienceDirect

BBA Clinical

journal homepage: www.elsevier.com/locate/bbaclin

Up-regulation of the human-specific CHRFAM7A gene in inflammatory bowel disease

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ARTICLE INFO

Article history:
Received 23 November 2015
Received in revised form 16 December 2015
Accepted 17 December 2015
Available online 8 January 2016

ABSTRACT

Background: The α7-subunit of the α7-nicotinic acetylcholine receptor (α7-nAChR) is an obligatory intermediate for the anti-inflammatory effects of the vagus nerve. But in humans, there exists a second gene called CHRFAM7A that encodes a dominant negative α7-nAChR inhibitor. Here, we investigated whether their expression was altered in inflammatory bowel disease (IBD) and colon cancer.
Methods: Quantitative RT-PCR measured gene expression of human α7-nAChR gene (CHRNA7), CHRFAM7A, TBC3D1, and actin in biopsies of normal large and small intestine, and compared to their expression in biopsies of ulcerative colitis, Crohn's disease, and colon cancer.
Results: qRT-PCR showed that CHRFAM7A and CHRNA7 gene expression was significantly ($p < .02$) up-regulated in IBD ($N = 64$). Gene expression was unchanged in colon cancer. Further analyses revealed that there were differences in ulcerative colitis and Crohn's Disease. Colon biopsies of ulcerative colitis ($N = 33$) confirmed increased expression of CHRFAM7A and decreased in CHRNA7 expression ($p < 0.001$). Biopsies of Crohn's disease ($N = 31$), however, showed only small changes in CHRFAM7A expression ($p < 0.04$) and no change in CHRNA7. When segregated by tissue source, both CHRFAM7A up-regulation ($p < 0.02$) and CHRNA7 down-regulation ($p < 0.001$) were measured in colon, but not in small intestine.
Conclusion: The human-specific CHRFAM7A gene is up-regulated, and its target, CHRNA7, down-regulated, in IBD. Differences between ulcerative colitis and Crohn's disease tie to location of disease.
Significance: The appearance of IBD in modern humans may be consequent to the emergence of CHRFAM7A, a human-specific α7-nAChR antagonist. CHRFAM7A could present a new, unrecognized target for development of IBD therapeutics.

1. Introduction

The emergence of human-specific genes (HSG) in the course of human evolution are presumed to have enabled the adaptation of humans to new environments and new behaviors [1–9] but their specific physiological functions are often unknown. The human-specific CHRFAM7A gene is a case in point. First, it encodes a uniquely human and independently regulated subunit of the α7-nicotinic acetylcholine receptor (α7-nAChR) that regulates neurotransmitter function. Therefore, it is presumed to affect CNS function. When co-expressed with α7-nAChR, however, CHRFAM7A is a dominant negative regulator of neurotransmitter binding to, and activation of, the α7-nAChR, thereby potentially altering the central nervous system functions of α7-nAChR in its regulation of processes like cognition, memory, and mental health [10–19].

But CHRFAM7A is also widely expressed in leukocytes and epithelial cells [11,19–21], where it is presumed to regulate the powerful anti-inflammatory effects of α7-nAChR activation [22–24]. Because the activation of α7-nAChR is an obligatory intermediate for vagus nerve control of inflammation [25], CHRFAM7A in humans must therefore regulate the anti-inflammatory vagus nerve. If so, it raises the possibility that CHRFAM7A expression in peripheral tissues [20,21] could be associated with human inflammatory disease like, for example, inflammatory bowel disease (IBDs).

IBDs have complex molecular etiologies of genetic, epigenetic, microbial, and environmental origin that present as highly heterogeneous episodes of gut inflammation [26,27], making animal modeling difficult [28]. Accordingly, the response of IBDs to behavioral, dietary, and therapeutic interventions is often enigmatic, as exemplified by both protective and deleterious effects of nicotine and nicotine withdrawal on its remission, recurrence, and treatment [26,27]. Here, we explored the

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http://dx.doi.org/10.1016/j.bbacli.2015.12.003

possibility that a concomitant, but differentially regulated [20,21] expression of the human-specific and pro-inflammatory CHRFAM7A gene and its anti-inflammatory α7-nAChR target [11,19,24,25] (CHRNA7), could be implicated in IBD.

2. Materials and methods

2.1. Biopsies of inflammatory bowel disease and colon cancer

cDNAs in OriGene TissueScan Arrays from characterized biopsies of ulcerative colitis (CCRT101 and CCRT 102), Crohn's disease (CCRT101 and CCRT 102), or colon cancer (HCRT104) were used to assess gene expression in disease (N = 109) and control (N = 19) tissue biopsies. All characteristics of these specimens are available online with detailed clinical information, histology slides of each biopsy, and the quality control data for RNA isolation and cDNA preparations at California http://www.origene.com/qPCR/Tissue-qPCR-Arrays.aspx. The original de-identified tissues were collected from accredited medical institutions in the United States using IRB-approved protocols, selected by board-certified pathologists and then deposited into the OriGene tissue biorepository along with all of the available clinical data supporting the pathology diagnoses. The specific array plates used contained cDNA synthesized prepared from RNA extracted from these pathologist-verified tissues. The quantity of cDNA was normalized, first validated with ß-actin at OriGene and the findings replicated in the course of the gene expression studies described here. The Ct value of actin gene expression in each well was determined in our laboratories was highly consistent (average 20.89 cycles ± 0.1 (SEM, N = 135)) and the individual values from each well used to calculate relative gene expression in each biopsy using the actin primers, as specifically noted by the array manufacturer.

2.2. PCR, primers, and the conditions for CHRFAM7A, CHRNA7, and TBC1D3 analyses

The PCR reaction was performed in 50 μl containing 45 μl PCR blue mix (Invitrogen), 1 μl of each primer (10 μM), 300 ng cDNA, and 2 μl water. The cycling conditions were 94 °C for 4 min followed by 35 cycles of 94 °C for 30 s, 60 °C for 30 s, and 72 °C for 60 s, and a final extension at 72 °C for 5 min. Ten microliters of each PCR product was resolved on a 2% agarose gel and images were acquired using Alpha Innotech imaging system. Real-time qPCR was performed in a 25 μl reaction containing 12.5 μl 2× CYBR Green PCR Master Mix (BioRad), 0.5 μl of each primer (10 μM), 1 μl cDNA, and 10.5 μl water. PCR cycling conditions were 95 °C for 10 min followed by 45 cycles of 94 °C for 25 s, 60 °C for 25 s, and 72 °C for 40 s. Primer efficiency for CHRFAM7A and CHRNA7 were 100% and 94%, respectively.

Table 1
Tissue biopsies.

	Ulcerative colitis	Crohn's disease	Colon cancer			
Array ID	CCRT101/102	CCRT101/102	HCRT104			
Biopsies studied	44	42	48			
Normal	11		8			
Disease	33	31	40			
Control biopsies						
Location of lesion	Colon 8 Small Intestine 3		Colon 8			
Gender						
Male	7		2			
Female	4		6			
Age (years)	54 (26–89)		78 (60–89)			
Male	56 (26–89)		82 (81–82)			
Female	50 (29–70)		77 (60–89)			
% Mucosa	48% (10–90)		N/A			
Male	50% (20–85)		N/A			
Female	44% (10–90)		N/A			
Disease biopsies			Tissue	Stage		N
Location of lesion	Colon 33	Colon 13	Colon	I		5
	Small Intestine 0	Small Intestine 18	Colon	II		9
			Colon	III		16
			Colon	IV		10
Gender						
Male	21	14		16		
Female	12	17		24		
Age (years)	39 (22–76)	38 (19–65)		68 (21–89)		
Male	38 (22–72)	40 (20–64)		65 (21–82)		
Female	45 (26–76)	36 (19–65)		70 (45–89)		
			Well	Moderate	Poor	Un-diff.
% Mucosa/differentiation*	43% (10–100)	38 (0–00)	10	19	6	5
Male	43% (10–100)	41 (0–80)	7	11	4	2
Female	43% (10–95)	35 (10–90)	3	8	2	3
Pathology						
Lesion/tumor (%)	100		71% (25–95%)			
Male	100		69.9 (25–95)			
Female	100		73.7 (40–90)			
Hypercellular stroma (%)	N/A		16% (0–55%)			
Male	N/A		17.3 (0–55%)			
Female	N/A		14.4 (0–35%)			
Hypocellular Stroma (%)	N/A		1.95% (0–28%)			
Male	N/A		2.7 (0–28%)			
Female	N/A		0.9 (0–10%)			
Necrosis (%)	0		4.9% (0–40%)			
Male	0		3.6% (0–40%)			
Female	0		6.6 (0–20%)			