HHS Public Access

Author manuscript

RNA Dis. Author manuscript; available in PMC 2015 May 22.

Published in final edited form as:

RNA Dis. 2015 ; 2(1): . doi:10.14800/rd.472.

MicroRNA-133α regulates neurotensin-associated colonic inflammation in colonic epithelial cells and experimental colitis

Ivy Ka Man Law and **Charalabos Pothoulakis**

Inflammatory Bowel Disease Center, Division of Digestive Diseases, David Geffen School of Medicine at UCLA, Los Angeles, California, USA

Abstract

Ulcerative colitis (UC) and Crohn's Disease (CD) are the two most common forms of Inflammatory Bowel Diseases (IBD) marked by chronic and persistent inflammation. Neurotensin (NT), together with its receptor, NT receptor 1 (NTR1), are important mediators in intestinal inflammation and their expression is upregulated in the intestine of experimental colitis models and UC colonic biopsies. MicroRNAs (miRNAs) are short, non-coding RNA molecules which act as transcription repressors. We have previously shown that NT exposure upregulates miR-133α expression in human colonocytes NCM460 cells overexpressing NTR1 (NCM460-NTR1). Recently, miR-133α was further examined forits role in NT-associated proinflammatory signaling cascades and acute colitis *in vivo*. Our study shows that NT-induced miR-133α upregulation modulates NF-κB phosphorylation and promotes proinflammatory cytokine production. In addition, intracolonicinjection of antisense-miR-133α before colitis induction improves histological scores and proinflammatory cytokine transcription. More importantly, dysregulation of miR-133α levels and aftiphilin (AFTPH), a newly-identified miR-133α downstream target, is found only in UC patients, but not in patients with CD. Taken together, we identified NTR1/miR-133α/aftiphilin as a novel regulatory axis involved in NT-associated colonic inflammation in human colonocytes, acute colitis mouse model and in colonic biopsies from UC patients. Our results also provide evidence that colonic levels of NTR1, miR-133α and aftiphilin may also serve as potential biomarkers in UC.

Keywords

inflammatory bowel disease; experimental colitis; neurotensin; miR-133α; aftiphilin

Ulcerative colitis (UC) and Crohn's disease (CD) are two most common forms of Inflammatory Bowel Diseases (IBD), which are characterized by chronic and persistent inflammation with relapsing and remitting episodes. Neurotensin (NT) is a proinflammatory neuropeptide found in the central nervous system and the intestine [1, 2], while NT receptor 1 (NTR1), which has a high affinity with NT, is expressed in neurons [3], colonic epithelial [2, 4–6], immune cells [7, 8] and colon cancer cell lines [9]. We and others have shown that during intestinal inflammation in experimental colitis mouse models [2, 5, 10, 11]

Correspondence: Charalabos Pothoulakis, cpothoulakis@mednet.ucla.edu.

and in the colon of UC patients [5], colonic NT and NTR1 expression is upregulated. Importantly, NT/NTR1 signaling promotes inflammation in colons in animal models induced with acute colitis [2,4], while mice deficient in NTR1 have attenuated colitis development and reduced mortality [4, 12] during the induction of experimental colitis. In addition, NT/NTR1 signaling has been shown to target on ERK and Akt activation in human colonic epithelial cells [6, 9, 13]. The above evidence suggests that NT/NTR1 signaling is important in colonic inflammation both in colitis models and in human pathophysiology.

MicroRNAs (miRNAs) are short, non-coding RNA molecules which act as transcription repressors. They represent targets of inflammatory agents and act as regulators of genes responsible for immune response [14–18]. Several studies demonstrated differential miRNA expression in the circulation [19–22] and in the colonic mucosa of UC [23–26] and CD [25, 27] patients as well as in the colon of experimental colitis mouse models [24]. However, whether miR-133α was involved in the development and progress of colitis was not known. Recently, we have shown that the binding of NT to NTR1 in human colonocytes NCM460 cells overexpressing NTR1 (NCM460-NTR1) modulates expression levels of several miRNAs[9], including miR-133α. Interestingly, during colon cancer development, miR-133α and its target genes regulate activation of ERK [28, 29] and Akt [30, 31] which are also targeted by NTR1 signaling [6, 9, 13].

In our recent study, we investigated how miR-133α was involved in NT-associated colonic inflammation. We first examined miR-133α levels in two human colon epithelial cell lines, the non-transformed NCM460 cells (expressing low NTR1 levels) overexpressing NTR1 (NCM460-NTR1) and the colon cancer HCT116 cells expressing high NTR1 levels. Consistent with our previous findings [9], miR-133α expression was upregulated upon NT exposure in both cell lines. We then studied the association of miR-133α expression with colonic inflammation *in vivo*. NT was administered to wild type C57BL/6 mice through intracolonic administration as a mediator of acute colonic inflammation [2, 12]. RT-PCR analysis revealed that NT stimulation upregulated miR-133α expression in colon tissues in wild type mice. We also found that miR-133α expression was upregulated in two chemically induced mouse colitis models (2, 4, 6- trinitrobenzenesulfonic acid, TNBS; and dextran sodium sulfate, DSS). Together, these results imply that miR-133α may play a role in intestinal inflammation *in vitro* and *in vivo*.

Dysregulation of mucosal homeostasis in IBD is caused by prolonged inflammation in the colonic mucosa. Therefore, we next examined the role of miR-133α in proinflammatory signaling responses in human colonocytes *in vitro* and in acute experimental colitis *in vivo*. The differential signaling activated by NTR1 activation in human colonocyte NCM460-NTR1 cells was studied after downregulation of miR-133α *in vitro*. Our results showed that miR-133α not only regulated ERK activation as previously described [28,29], but miR-133α silencing also reduced NF-κB activation and proinflammatory cytokine production. Several studies have shown that localized delivery of antisense oligonucleotides against NF-κB [32], TNF alpha [33] or miR-141 [34] are effective in ameliorating experimental colitis. To examine the impact of miR-133α *in vivo* we reduced local colonic miR-133α levels by intracolonic injection of antisense-miR-133α oligonucleotides prior to acute colitis induction by TNBS administration. Mice administered with antisense-miR-133α oligonucleotides

showed improved histological score and reduced proinflammatory cytokine production. In addition, miR-133α level was mainly upregulated in colonic epithelial cells in mice with colitis and majority of antisense oligonucleotides delivered intracolonically was confined to colonic epithelial cells *in vivo* [34]. Taken together, our data showed that reducing miR-133α expression in colonic epithelial cells through local delivery of antisense- miR-133α oligonucleotides *in vivo* attenuates development of acute colitis.

As described above miRs inhibit transcription of different genes serving as downstream targets thereby affecting a wide range of diseases, including inflammation [35]. MiRs exert their effect by binding 3'untranslated regions (UTRs) of transcript [36], thereby repressing deadenylation during translation and promoting mRNA degradation [37]. Several downstream targets have been identified for miR-133α that play a role in inflammation (UCP2 [38]), cancer (EGFR [39,40], GSTP1 [41], PNP [42], TAGLN2 [42, 43]) and oxidative stress (GM-CSF [44]). Using *in silico* search in 3 online databases [miRBase (www.mirbase.org) and PicTar (http://pictar.mdc-berlin.de);TargetScanHuman (www.targetscan.org)], we have identified a novel miR-133α target, aftiphilin (AFTPH). NT stimulation of human colonocytes or miR-133α overexpression in these cells lowered AFTPH mRNA levels. The role of AFTPH in cellular function has been studied in a small number of studies. AFTPH contains binding motifs for the structural protein clathrin [45] and is localized in *trans*-golgi network (TGN) [46]. Although TGN morphology is not affected by AFTPH gene silencing [47], downregulated AFTPH levels promote dysregulated exocytosis of Weibel-Palade bodies in endothelial cells [48].

The clinical relevance of our findings was also examined by measuring the levels of miR-133α and its novel downstream target, AFTPH, in colonic cDNA samples from UC and CD patients and their normal controls. We showed that increased miR-133α, but reduced AFTPH transcription were detected in mucosal biopsies of UC, but not CD patients, when compared to normal controls. In addition, we have also investigated the role of AFTPH, a protein responsible for intracellular trafficking [45] and exocytosis [48], in proinflammatory signaling cascades. Consistent with the results from our miR-133α studies, AFTPH gene-silencing *in vitro* enhanced NF-κB phosphorylation and IL-1β production in human colonocytes. Therefore, dysregulation in miR-133α and AFTPH expression modulates proinflammatory responses in human colonocytes and possibly in the mucosa in colons during colitis and IBD.

One of the important strategies of drug development in IBD is to limit chronic inflammation. Understanding the mechanism(s) of IBD-related inflammation enables us to target new genes and /or signaling pathways. Our recent study identified a novel NTR1/miR-133α/ AFTPH network involved in proinflammatory signaling regulation in colonic epithelial cells *in vitro* and *in vivo*, present evidence implying their potential use as biomarkers for UC. Consequently, future work will explore the effect of reducing AFTPH expression in acute colitis development by intracolonic administration of siRNA against AFTPH. In addition, although MyoD and myogenin are the two known transcription factors promoting miR-133α transcription during muscle development [49, 50], the molecular mechanisms for regulating miR-133α during inflammation has not been examined. Studies on the mechanism(s) related

to the regulation of expression of miR-133α will provide more insights to the proinflammatory response during colitis.

Acknowledgements

This work was supported by NIH grant DK60729, the Neuroendocrine Assay Core, NIDDK P50 DK 64539, the Eli and Edythe Broad Research Foundation, the Blinder Research Foundation for Crohn's Disease, and the Crohn's and Colitis Foundation of America, Inc.

References

1. Polak JM, Sullivan SN, Bloom SR, Buchan AM, Facer P, Brown MR, et al. Specific localisation of neurotensin to the n cell in human intestine by radioimmunoassay and immunocytochemistry. Nature. 1977; 270:183–184. [PubMed: 337160]
2. Castagliuolo I, Wang CC, Valenick L, Pasha A, Nikulasson S, Carraway RE, et al. Neurotensin is a proinflammatory neuropeptide in colonic inflammation. J Clin Invest. 1999; 103:843–849. [PubMed: 10079105]
3. Fassio A, Evans G, Grisshammer R, Bolam JP, Mimmack M, Emson PC. Distribution of the neurotensin receptor nts1 in the rat cns studied using an amino-terminal directed antibody. Neuropharmacology. 2000; 39:1430–1442. [PubMed: 10818259]
4. Koon HW, Kim YS, Xu H, Kumar A, Zhao D, Karagiannides I, et al. Neurotensin induces il-6 secretion in mouse preadipocytes and adipose tissues during 2,4,6,-trinitrobenzensulphonic acid-induced colitis. Proc Natl Acad Sci U S A. 2009; 106:8766–8771. [PubMed: 19443690]
5. Brun P, Mastrotto C, Beggiao E, Stefani A, Barzon L, Sturniolo GC, et al. Neuropeptide neurotensin stimulates intestinal wound healing following chronic intestinal inflammation. Am J Physiol Gastrointest Liver Physiol. 2005; 288:G621–G629. [PubMed: 15764810]
6. Zhao D, Keates AC, Kuhnt-Moore S, Moyer MP, Kelly CP, Pothoulakis C. Signal transduction pathways mediating neurotensin-stimulated interleukin-8 expression in human colonocytes. J Biol Chem. 2001; 276:44464–44471. [PubMed: 11574537]
7. Sundman L, Saarialho-Kere U, Vendelin J, Lindfors K, Assadi G, Kaukinen K, et al. Neuropeptide s receptor 1 expression in the intestine and skin--putative role in peptide hormone secretion. Neurogastroenterol Motil. 2010; 22:79–87. e30. [PubMed: 19614867]
8. da Silva L, Neves BM, Moura L, Cruz MT, Carvalho E. Neurotensin downregulates the pro-inflammatory properties of skin dendritic cells and increases epidermal growth factor expression. Biochimica et Biophysica Acta (BBA) - Molecular Cell Research. 2011; 1813:1863–1871.
9. Bakirtzi K, Hatziapostolou M, Karagiannides I, Polytarchou C, Jaeger S, Iliopoulos D, et al. Neurotensin signaling activates microrna-21 and-155 and akt, promotes tumor growth in mice, and is increased in human colon tumors. Gastroenterology. 2011; 141:1749–1761. [PubMed: 21806946]
10. Margolis KG, Gershon MD. Neuropeptides and inflammatory bowel disease. Curr Opin Gastroenterol. 2009; 25:503–511. [PubMed: 19816171]
11. Zhao D, Pothoulakis C. Effects of nt on gastrointestinal motility and secretion, and role in intestinal inflammation. Peptides. 2006; 27:2434–2444. [PubMed: 16872719]
12. Bugni JM, Rabadi LA, Jubbal K, Karagiannides I, Lawson G, Pothoulakis C. The neurotensin receptor-1 promotes tumor development in a sporadic but not an inflammation-associated mouse model of colon cancer. Int J Cancer. 2012; 130:1798–1805. [PubMed: 21630261]
13. Zhao D, Bakirtzi K, Zhan Y, Zeng H, Koon HW, Pothoulakis C. Insulin-like growth factor-1 receptor transactivation modulates the inflammatory and proliferative responses of neurotensin in human colonic epithelial cells. J Biol Chem. 2011; 286:6092–6099. [PubMed: 21212273]
14. Bhattacharyya S, Sen P, Wallet M, Long B, Baldwin AS Jr, Tisch R. Immunoregulation of dendritic cells by il-10 is mediated through suppression of the pi3k/akt pathway and of i{kappa}b kinase activity. Blood. 2004; 104:1100–1109. [PubMed: 15113757]
15. Chiou TJ. The role of micrornas in sensing nutrient stress. Plant Cell Environ. 2007; 30:323–332. [PubMed: 17263777]

16. Marsit CJ, Eddy K, Kelsey KT. Microrna responses to cellular stress. Cancer Res. 2006; 66:10843–10848. [PubMed: 17108120]
17. Ahmed EA, van der Vaart A, Barten A, Kal HB, Chen J, Lou Z, et al. Differences in dna double strand breaks repair in male germ cell types: Lessons learned from a differential expression of mdc1 and 53bp1. DNA Repair (Amst). 2007; 6:1243–1254. [PubMed: 17376750]
18. Asirvatham AJ, Gregorie CJ, Hu Z, Magner WJ, Tomasi TB. Microrna targets in immune genes and the dicer/argonaute and are machinery components. Mol Immunol. 2008; 45:1995–2006. [PubMed: 18061676]
19. Wu F, Guo NJ, Tian H, Marohn M, Gearhart S, Bayless TM, et al. Peripheral blood micrornas distinguish active ulcerative colitis and crohn's disease. Inflammatory Bowel Diseases. 2011; 17:241–250. [PubMed: 20812331]
20. Zahm AM, Thayu M, Hand NJ, Horner A, Leonard MB, Friedman JR. Circulating microrna is a biomarker of pediatric crohn disease. J Pediatr Gastroenterol Nutr. 2011; 53:26–33. [PubMed: 21546856]
21. Paraskevi A, Theodoropoulos G, Papaconstantinou I, Mantzaris G, Nikiteas N, Gazouli M. Circulating microrna in inflammatory bowel disease. J Crohns Colitis. 2012; 6:900–904. [PubMed: 22386737]
22. Duttagupta R, DiRienzo S, Jiang R, Bowers J, Gollub J, Kao J, et al. Genome-wide maps of circulating mirna biomarkers for ulcerative colitis. PLoS ONE. 2012; 7:e31241. [PubMed: 22359580]
23. Wu F, Zikusoka M, Trindade A, Dassopoulos T, Harris ML, Bayless TM, et al. Micrornas are differentially expressed in ulcerative colitis and alter expression of macrophage inflammatory peptide-2 alpha. Gastroenterology. 2008; 135:1624–1635. [PubMed: 18835392]
24. Bian Z, Li L, Cui J, Zhang H, Liu Y, Zhang CY, et al. Role of mir-150-targeting c-myb in colonic epithelial disruption during dextran sulphate sodium-induced murine experimental colitis and human ulcerative colitis. J Pathol. 2011; 225:544–553. [PubMed: 21590770]
25. Fasseu M, Treton X, Guichard C, Pedruzzi E, Cazals-Hatem D, Richard C, et al. Identification of restricted subsets of mature microrna abnormally expressed in inactive colonic mucosa of patients with inflammatory bowel disease. PLoS ONE. 2010; 5:e13160. [PubMed: 20957151]
26. Takagi T, Naito Y, Mizushima K, Hirata I, Yagi N, Tomatsuri N, et al. Increased expression of microrna in the inflamed colonic mucosa of patients with active ulcerative colitis. Journal of Gastroenterology and Hepatology. 2010; 25:S129–S133. [PubMed: 20586854]
27. Wu F, Zhang S, Dassopoulos T, Harris ML, Bayless TM, Meltzer SJ, et al. Identification of micrornas associated with ileal and colonic crohn's disease. Inflamm Bowel Dis. 2010; 16:1729–1738. [PubMed: 20848482]
28. Li Q, Lin X, Yang X, Chang J. Nfatc4 is negatively regulated in mir-133a-mediated cardiomyocyte hypertrophic repression. Am J Physiol Heart Circ Physiol. 2010; 298:H1340–H1347. [PubMed: 20173049]
29. Feng Y, Niu LL, Wei W, Zhang WY, Li XY, Cao JH, et al. A feedback circuit between mir-133 and the erk1/2 pathway involving an exquisite mechanism for regulating myoblast proliferation and differentiation. Cell Death Dis. 2013; 4:e934. [PubMed: 24287695]
30. Josse C, Bouznad N, Geurts P, Irrthum A, Huynh-Thu VA, Servais L, et al. Identification of a microrna landscape targeting the pi3k/akt signaling pathway in inflammation-induced colorectal carcinogenesis. Am J Physiol Gastrointest Liver Physiol. 2014; 306:G229–G243. [PubMed: 24464560]
31. Cui W, Zhang S, Shan C, Zhou L, Zhou Z. Microrna-133a regulates the cell cycle and proliferation of breast cancer cells by targeting epidermal growth factor receptor through the egfr/akt signaling pathway. FEBS J. 2013; 280:3962–3974. [PubMed: 23786162]
32. Murano M, Maemura K, Hirata I, Toshina K, Nishikawa T, Hamamoto N, et al. Therapeutic effect of intracolonically administered nuclear factor kappa b (p65) antisense oligonucleotide on mouse dextran sulphate sodium (dss)-induced colitis. Clin Exp Immunol. 2000; 120:51–58. [PubMed: 10759763]

33. Zuo L, Huang Z, Dong L, Xu L, Zhu Y, Zeng K, et al. Targeting delivery of anti-tnfalpha oligonucleotide into activated colonic macrophages protects against experimental colitis. Gut. 2010; 59:470–479. [PubMed: 19951904]
34. Huang Z, Shi T, Zhou Q, Shi S, Zhao R, Shi H, et al. Mir-141 regulates colonic leukocytic trafficking by targeting cxcl12β during murine colitis and human crohn's disease. Gut. 2013; 63:1247–1257. [PubMed: 24000293]
35. Contreras J, Rao DS. Micrornas in inflammation and immune responses. Leukemia. 2012; 26:404–413. [PubMed: 22182919]
36. McKenna LB, Schug J, Vourekas A, McKenna JB, Bramswig NC, Friedman JR, et al. Micrornas control intestinal epithelial differentiation, architecture, and barrier function. Gastroenterology. 2010; 139:1654–1664. [PubMed: 20659473]
37. Bartel DP. Micrornas: Target recognition and regulatory functions. Cell. 2009; 136:215–233. [PubMed: 19167326]
38. Bandyopadhyay S, Lane T, Venugopal R, Parthasarathy PT, Cho Y, Galam L, et al. Microrna-133a-1 regulates inflammasome activation through uncoupling protein-2. Biochem Biophys Res Commun. 2013; 439:407–412. [PubMed: 23988448]
39. Zhou Y, Wu D, Tao J, Qu P, Zhou Z, Hou J. Microrna-133 inhibits cell proliferation, migration and invasion by targeting epidermal growth factor receptor and its downstream effector proteins in bladder cancer. Scand J Urol. 2013; 47:423–432. [PubMed: 23206218]
40. Tao J, Wu D, Xu B, Qian W, Li P, Lu Q, et al. Microrna-133 inhibits cell proliferation, migration and invasion in prostate cancer cells by targeting the epidermal growth factor receptor. Oncol Rep. 2012; 27:1967–1975. [PubMed: 22407299]
41. Uchida Y, Chiyomaru T, Enokida H, Kawakami K, Tatarano S, Kawahara K, et al. Mir-133a induces apoptosis through direct regulation of gstp1 in bladder cancer cell lines. Urol Oncol. 2013; 31:115–123. [PubMed: 21396852]
42. Nohata N, Hanazawa T, Kikkawa N, Sakurai D, Sasaki K, Chiyomaru T, et al. Identification of novel molecular targets regulated by tumor suppressive mir-1/mir-133a in maxillary sinus squamous cell carcinoma. Int J Oncol. 2011; 39:1099–1107. [PubMed: 21701775]
43. Yoshino H, Chiyomaru T, Enokida H, Kawakami K, Tatarano S, Nishiyama K, et al. The tumour-suppressive function of mir-1 and mir-133a targeting tagln2 in bladder cancer. Br J Cancer. 2011; 104:808–818. [PubMed: 21304530]
44. Sturrock A, Mir-Kasimov M, Baker J, Rowley J, Paine R 3rd. Key role of microrna in the regulation of granulocyte-macrophage colony stimulating factor expression in murine alveolar epithelial cells during oxidative stress. J Biol Chem. 2013; 289:4095–4105. [PubMed: 24371146]
45. Mattera R, Ritter B, Sidhu SS, McPherson PS, Bonifacino JS. Definition of the consensus motif recognized by gamma-adaptin ear domains. J Biol Chem. 2004; 279:8018–8028. [PubMed: 14665628]
46. Burman JL, Wasiak S, Ritter B, de Heuvel E, McPherson PS. Aftiphilin is a component of the clathrin machinery in neurons. FEBS Lett. 2005; 579:2177–2184. [PubMed: 15811338]
47. Hirst J, Borner GH, Harbour M, Robinson MS. The aftiphilin/p200/gamma-synergin complex. Mol Biol Cell. 2005; 16:2554–2565. [PubMed: 15758025]
48. Lui-Roberts WW, Ferraro F, Nightingale TD, Cutler DF. Aftiphilin and gamma-synergin are required for secretagogue sensitivity of weibel-palade bodies in endothelial cells. Mol Biol Cell. 2008; 19:5072–5081. [PubMed: 18815278]
49. Chen X, Wang K, Chen J, Guo J, Yin Y, Cai X, et al. In vitro evidence suggests that mir-133a-mediated regulation of uncoupling protein 2 (ucp2) is an indispensable step in myogenic differentiation. Journal of Biological Chemistry. 2009; 284:5362–5369. [PubMed: 19073597]
50. Rao PK, Kumar RM, Farkhondeh M, Baskerville S, Lodish HF. Myogenic factors that regulate expression of muscle-specific micrornas. Proc Natl Acad Sci U S A. 2006; 103:8721–8726. [PubMed: 16731620]