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Research paper

The expression profiling and ontology analysis of noncoding RNAs in peritoneal fibrosis induced by peritoneal dialysis fluid

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ABSTRACT

Increasing amounts of evidence have indicated that noncoding RNAs (ncRNAs) have important regulatory potential in various biological processes. However, the contributions of ncRNAs, especially long noncoding RNAs (lncRNAs), to peritoneal fibrosis remain largely unknown. The aim of this study was to investigate miRNA, lncRNA and mRNA expression profiles and their potential roles in the process of peritoneal fibrosis. Microarray expression profiles of the miRNAs, lncRNAs and mRNAs were determined in normal control peritoneum and in a mouse model of peritoneal dialysis fluid (PDF)-induced fibrotic peritoneum. Differential expression, pathway and gene network analyses were developed to identify possible functional RNA molecules in peritoneal fibrosis. Compared to the normal control, 232 lncRNAs (127 up-regulated and 105 down-regulated), 154 mRNAs (87 up-regulated and 67 down-regulated) and 15 miRNAs (14 miRNAs up-regulated and 1 down-regulated) were differentially expressed in the fibrotic peritoneum. Among the differentially expressed ncRNAs, 9 lncRNAs and 5 miRNAs were validated by real-time RT-PCR. Pathway analysis showed that the Jak-STAT, TGF-beta and MAPK signaling pathways had a close relationship with peritoneal fibrosis. Gene co-expression network analysis identified many genes, including JunB, HSP72, and Nedd9. It also identified lncRNAs AK089579, AK080622, and ENSMUST00000053838 and miRNAs miR-182 and miR-488. All of these species potentially play a key role in peritoneal fibrosis. Our results provide a foundation and an expansive view of the roles and mechanisms of ncRNAs in PDF-induced peritoneal fibrosis.

1. Introduction

Peritoneal fibrosis is a major and severe complication in patients receiving continuous ambulatory peritoneal dialysis (CAPD). Long-term exposure to peritoneal dialysis fluid (PDF) is a cause of progressive peritoneal fibrosis, resulting in reduced ultrafiltration across the peritoneal membrane that ultimately leads to withdrawal from CAPD in many patients (Yanez-Mo et al., 2003). Histological changes in the peritoneum in response to long-term PD treatment include a loss of the mesothelial cell monolayer, excessive deposition of extracellular matrix, and angiogenesis (Mateijsen et al., 1999; Williams et al., 2002). However, the mechanisms and effective anti-fibrosis therapies of peritoneal fibrosis induced by PDF remain largely undefined.

Non-coding RNAs (ncRNAs) are a type of RNA that do not code for proteins. They were previously regarded as "transcriptional noise" but are now known to have important regulatory potential in transcription and post-transcription. The most studied ncRNAs are the microRNAs (miRNAs), which are typically ~22 nt nucleotides long and negatively regulate target messenger RNAs (mRNAs) through the induction of mRNA degradation or translational inhibition. Accumulating evidence shows that miRNAs regulate diverse biological processes, including cell differentiation, proliferation, and apoptosis, and that aberrant expression of miRNAs may lead to severe diseases (Ambros, 2004; Krol et al., 2010). The miRNAs miR-21, miR-30a, miR-155 and miR-29 have been confirmed to be involved in fibrosis (Wang et al., 2012; Bhattacharyya et al., 2013; Yamada et al., 2013; Zhou et al., 2013). Furthermore, miR-29 and miR-30a were reported to have the ability to ameliorate peritoneal fibrosis in animal models of PD (Zhou et al., 2013; Yu et al., 2014), However, more research is needed to elucidate the complicated relationship between miRNAs and peritoneal fibrosis.

Long non-coding RNAs (lncRNAs), which are defined as ncRNAs ranging in length from 200 nt to ~100 kb, have become an area of increased research focus (Lee, 2012). They have been shown to exert comprehensive effects on biological processes through a variety of mechanisms (Mercer et al., 2009; Hu et al., 2012) and are thought to

Abbreviations: CAPD, continuous ambulatory peritoneal dialysis; PDF, peritoneal dialysis fluid; ncRNAs, non-coding RNAs; miRNAs, microRNAs; lncRNAs, long non-coding RNAs; ceRNA, competitive endogenous RNAs; MREs, miRNA response elements; EMT, epithelial-to-mesenchymal transition.

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Table 1
Primers for selected lncRNA.

LncRNA	Forward primer	Reverse primer
uc007eib.1	TGGTGTTCTGCGTGATGAGG	GAATAACACTTCCACCACAGG
ENSMUST00000053838	ACCAACCTGCTTGTCAGAG	CCTTGCGATTCTATTAGTCAGC
AK142426	TGGTGTTCTGCGTGATGAGG	GCTGTGTCTCAGTTCAAGTTAGC
uc008pwj.1	GGAGTTGGGAAGAGTGTGTC	GATGTAGTAACGGTAGGCAGC
uc007dlv.1	GAAAGTCTTCTGGGTTGGG	GTCAAGCAGCAAGCCTTAG
AK080622	TTCACTTGGAACCTCAGGC	CACTAACTGCTCCTTCGGTCTA
BC049991	TCTCCTTCATCCCTCTCCA	TGAGCACTCTACCAACGGA
AV310809	CCACTAACCTTCCCTATTACAT	AACTAATGGGTGCTGTGC
AK089579	GAGTGTCAGTTGGTGGAAAC	CTTCTTACCTGTGTGTTCTGC

play important roles in the pathophysiology of several diseases, including cancer, pulmonary fibrosis and cardiovascular disease (Cao et al., 2013; Yang et al., 2013; Liu et al., 2014). Moreover, in recent studies, lncRNAs have been discovered that can act as competitive endogenous RNAs (ceRNA) of miRNAs by sharing common miRNA response elements (MREs), inhibiting miRNA activity and attenuating the repression of miRNA-target genes (Ebert and Sharp, 2010; Poliseno et al., 2010; Cesana et al., 2011). Although lncRNA studies predominate in other fields, such as cancer biology, studies exploring the signature of lncRNA expression, the possible roles of lncRNAs, and the relationships with miRNAs and mRNAs in fibrosis biology remain limited.

In this study, the expression profiles of peritoneal lncRNAs, miRNAs and mRNAs were compared between normal control animals and a mouse model of peritoneal fibrosis induced by PDF. An integrative analysis combining the changes in the three groups of RNAs within different genetic networks was used to identify genes and pathways related to peritoneal fibrosis.

2. Materials and methods

2.1. Animals

BALB/c mice (male, 6–8 weeks of age) were provided by the Nanchang University Experimental Animal Center, Nanchang, China. A total of 36 BALB/c mice were randomly divided into 2 groups (n = 18 in each group): a normal control group and a peritoneal fibrosis model group. All animal experiments were carried out in accordance with the principles of the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

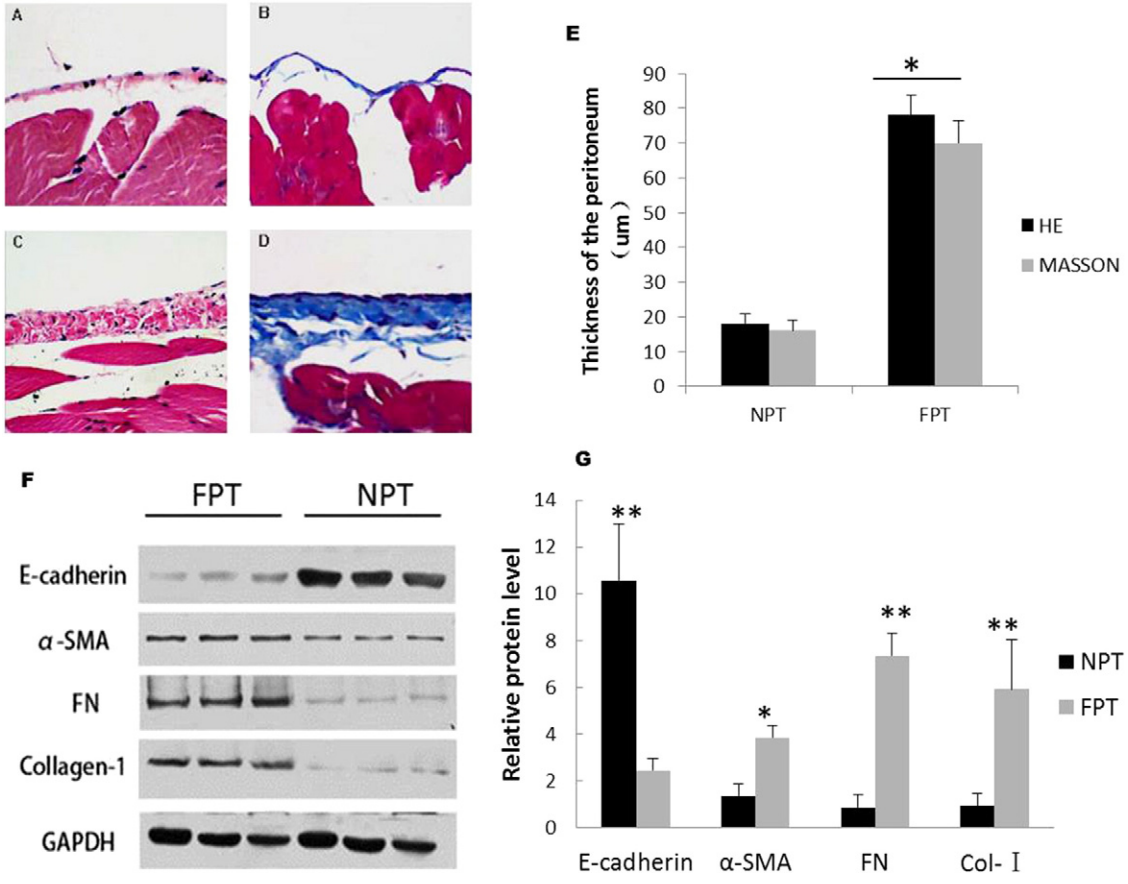

Fig. 1. Pathological changes of the peritoneum in a mouse model of PD. Severe peritoneal fibrosis developed in the mouse model of PD. Compared to the normal control (A: HE staining and B: Masson's trichrome staining), the peritoneum in the model group (C: HE staining and D: Masson's trichrome staining) was markedly thickened and showed remarkable proliferation of the collagen fibers. E: The peritoneum was significantly thicker in the model group. F, G: Western blot analysis showed that E-cadherin was decreased. In contrast, the expression level of α-SMA, collagen I and fibronectin were increased in the peritoneum of the mouse model of PD. NPT, normal peritoneal tissue; FPT, fibrosis peritoneal tissue; FN, fibronectin. *P < 0.05 when compared with NPT. **P < 0.01 when compared with NPT. Original magnification, ×200 (A–D).