Mesangial cells (MCs) are one of the resident cell types that have multiple functions in the renal glomerular capillary, including structural support of the capillary tuft, modulation of glomerular hemodynamics, and a phagocytosis. However, MCs are also involved in the pathogenesis of many glomerular disorders. During the response to insults of glomerula, the mesangial matrix expands and produces cytokines and chemokines that stimulate proliferation of MCs and cause infiltration of inflammatory cells in the mesangium. This response is a hallmark characteristic of many types of glomerulonephritis, including IgA nephropathy, mesangial proliferative glomerulonephritis, lupus nephritis, and diabetic nephropathy. MC proliferation is often accompanied by diminished
Therefore, it is essential to determine the agents that potentially inhibit MC proliferation while inducing apoptosis of these cells. The inhibition of cell proliferation is tightly linked to the mechanisms that regulate cell cycle progression. In dividing mammalian cells, cell cycle progression is regulated by a series of checkpoints and transitions in which temporal order is imposed by cyclin dependent kinases (CDKs) and cyclin D, acting in concert with their regulatory subunits. Cyclin kinase inhibitors (CKIs), including p21 and p27, negatively regulate the cell cycle by inhibiting the formation or activation of cyclin D1-CDK complexes. Thus cyclin D1, CDK2, p21 and p27 may serve as novel therapeutic targets through inhibiting proliferation and promoting apoptosis of MCs.

Previous studies revealed that administering Qufeng Tongluo Prescription (祛风通络方, QFTL) could reduce proteinuria/24 h, decrease the levels of blood-lipid, blood hypercoagulability, Ang II, the expressions of collagen IV (Col IV), fibronectin (FN), laminin (LN), transforming growth factor-β 1 (TGF-β 1) in renal tissue and alleviate the symptoms of glomerular sclerosis in rats. However, the effects and the regulatory mechanism of QFTL on the proliferation and apoptosis of MCs are still unclear. This study investigated whether QFTL could inhibit lipopolysaccharide (LPS)-stimulated proliferation and induce apoptosis of MCs and the underlying regulatory mechanism.

METHODS

Animals and MC

Health male Sprague–Dawley (SD) rats (120 ± 20 g) were purchased from Lab Animal Centre of Medical School of Xi’an Jiaotong University (certificate number: 2008016, SPF level). The MCs of rats were obtained from National Centre for Cell Preservation of Wuhan University.

Reagents and Drugs

LPS was purchased from Sigma (St. Louis, USA). Methyl thiazolyl tetrazolium (MTT) was purchased from Invitrogen Corp (Carlsbad, CA, USA). Benazepril hydrochloride tablets (10 mg tablet) were purchased from Novartis (Switzerland batch number 05039). QFTL was provided by the Pharmaceutical Facility of the Second Affiliated Hospital of Medical School of Xi’an Jiaotong University. QFTL consisted of Radix Astragali, Radix Rehmanniae, Sect. Sabia, Piper kadsura (Chiosy) Ohwi, Bungarus multicinctus, Zoacyx dhumnades, and Herba Taxilli. To condense with 1.5 g crude drugs contained in every milliliter of the decoction, and preserved it at 4 °C.

Preparation of Drug Serum

After one-week acclimatization bred, 30 SD male rats were randomly divided into the normal control group, Benazepril group and QFTL group (n=10 per group). Benazepril group was administered with 5.0 mg/(kg •d) of Benazepril, QFTL group with 3.75 g/(kg •d) of QFTL and normal control group with saline of the same volume. This treatment lasted for 7 days. After the last time of administration (before the administration absolving diet, but not drinking for 12 h) for 1 h, the rats were anesthetized with 3% pentobarbital (1.5 mL/kg) by abdominal cavity injection, and their blood was collected from abdominal aorta, centrifuged for 10 min at 3,000 × g for serum segregation, deactivated (aqueous bath at 56 °C for 30 min, filtered with a 0.22 μm micropore film. The serum samples were stored at –20 °C.

Resuscitation and Culture of Rat MCs

Frozen MCs were rapidly thawed (within 1 min) in 37 °C waterbath. The thawed MCs were transferred into a centrifuge tube, resuspended in 9 mL culture media [20% fetal calf serum-Dulbecco’s modified Eagle’s medium (FBS-DMEM)], and centrifuged at 1,000 × g for 5 min to rid of dimethyl sulfoxide (DMSO). The cell pellet was resuspended in 1 mL culture media to make the MCs suspension, adjusted at a density of 1 × 10^6 cells per mL, seeded into the 6-well plates, incubated to 80% confluency. The culture was subsequently passaged five times for the experiments. For each passage, the cells were washed one time with D-Hanks, and then digested with 2 mL of 0.25% pancreatic enzyme for 5 min at 37 °C.

Culture of MCs and Experimental Design

The MCs used in this experiment had undergone 5 passages and were cultured in RPMI-1640 supplemented with 20% fetal calf serum to make the cell suspension, adjusted at a density of 1 × 10^6 cells per mL, seeded into the 6-well plates, incubated to 80% confluency. The MCs were cultured with serum-free medium in order to synchronize MCs at G0/G1