Selective RNA interference of Raptor/mTORC1 protects against the progression of intervertebral disc degeneration in a rat tail temporary static compression model

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Disclosures: NK (N), TY (N), MR (N), YT (Surgical Spine, Inc.), TT (N), YK (N), KM (N), HO (N), TM (N), KKuroshima (N), YH (N), RK (N), KKakutani (Surgical Spine, Inc.)

INTRODUCTION:
Low back pain is a major global health problem, and intervertebral disc degeneration is recognized as one of the independent causes[1]. In intracellular signaling, the mammalian target of rapamycin (mTOR) is a serine/threonine kinase. The mTOR exists in the two complexes of mTOR complex 1 (mTORC1) containing regulatory-associated protein of mTOR (Raptor) and of mTOR complex 2 (mTORC2) containing rapamycin-insensitive companion of mTOR (Rictor). Autophagy, negatively controlled under the mTOR signaling pathway, is an intracellular clearance mechanism involved in maintaining cell homeostasis under stress conditions including nutrient deprivation and inflammation[2]. We hypothesized that mTOR signaling and autophagy would be essential for the disc, characterized as a low-nutrient organ. We previously reported that the *in-vitro* selective suppression of RAPTOR/mTORC1 by RNA interference (RNAi) could protect human disc cells with autophagy and Akt induction against apoptosis, senescence, and matrix catabolism[3]; however, the *in-vivo* effects remain undetermined. Therefore, an *in-vivo* study was designed to clarify effects of mTORC1 modulation by Raptor RNAi in a rat tail model of intervertebral disc degeneration induced by temporary static compression.

METHODS:
12-week-old male Sprague–Dawley rats were used (n = 60). (1) A preliminary experiment was performed to determine the injection volume. The contrast medium of 1.0–5.0 µl was injected using a 33-gauge needle to rat caudal discs from C7–C8 to C13–C14, respectively (n = 7), and computer tomographic images were taken immediately. Time-dependent disappearance of the contrast was also observed. (2) To confirm *in-vivo* successful transfection, intradiscal injection of an *in-vivo* lipofection agent with Alexa Fluor 555-labeled Raptor siRNA was performed. Mid-sagittal sections of the functional spinal unit were assessed by immunofluorescence at 7 and 56 d after injection. In addition, Western blotting for mTOR signaling was performed to assess the transfection efficiency of Raptor RNAi and altered cellular health including autophagy (marker LC3-II and substrate p62/SQSTM1), apoptosis (marker cleaved PARP), and senescence (marker p16/INK4A). (3) A rat tail model of disc degeneration induced by temporary static compression was designed[4]. Rat tails were affixed with an Ilizarov-type apparatus with springs between the 8th and 10th coccygeal (C) vertebrae. Non-specific siRNA was injected into C9–C10 (loaded control) and C12–C13 (unloaded control) discs, and Raptor siRNA was injected into C8–C9 (loaded experimental) and C11–C12 (unloaded experimental) discs. Then, axial force of 1.3 MPa was applied for 24 h and subsequently released. Radiographic, histological (Safranin-O staining), and immunofluorescent assessments were performed at 0–56 d after compression. (4) Alternatively, to further clarify the efficacy of Raptor RNAi on degenerated discs, Raptor siRNA was injected into discs that underwent distinct degeneration at 28 d after 24-h temporary compression. Radiographic, histological (Safranin-O staining), and immunofluorescent assessments were performed at 0 and 28 d after injection.

RESULTS:
(1) The contrast agent filled the nucleus pulposus (NP) region up to 2.0 µl, stained the annulus fibrosus at 2.5 µl, and leaked from the puncture site from ≥3.0 µl; therefore, 2.0-µl amount was selected for the following experiments (Fig. 1). The contrast was completely washed out in 12 hours after injection. (2) Immunofluorescence detected red signals for Alexa Fluor 555-labeled Raptor siRNA in discs 7 and even 56 d after RNAi. Positive red fluorescence was observed in the cytoplasm of disc cells. Western blotting also displayed prolonged decreases in Raptor protein expression at 7 and 56 d (7 d, 54.3%, $p <$ 0.01; 56 d, 60.4%, $p < 0.01$). Furthermore, western blotting showed autophagy induction by Raptor RNAi with increased LC3-II and decreased p62/SQSTM and decreased cleaved PARP and p16/INK4a (Fig. 2). (3) Radiographic disc height measurements presented no statistical difference between loaded Raptor siRNA-injected discs and loaded control siRNA-injected discs at 0, 7, 14 and 28 d (7 d, $p = 0.61$; 14 d, $p = 0.21$; 28 d, $p = 0.08$) but significantly maintained disc height in Raptor siRNA-injected disc at 56 d (61.1% versus 46.0%, $p < 0.01$). Histological grading showed less degenerative changes in loaded Raptor siRNA-injected discs compared to loaded control discs (6.3 points versus 10.7 points, $p < 0.01$) (Fig. 3). Immunofluorescence showed the lower percentage of apoptotic TUNEL-positive cells in loaded Raptor siRNA-injected discs at 56 d. (4) Although radiographic disc height measurements showed no significant difference between Raptor siRNA-injected discs and control siRNA-injected discs at 0 and 28 d, histological grading showed the trend toward reduced degeneration in loaded Raptor siRNA-injected discs (28 d, 8.0 points versus 9.2 points, $p = 0.09$).

DISCUSSION: *In-vivo* intradiscal RNAi knockdown of Raptor demonstrated enhanced cellular autophagy, inhibited apoptotic cell death and senescent cell aging, radiographic height loss, and histological degeneration, indicating disc cell and matrix-protective effects. On the other hand, for degenerated discs induced by prior compressive load, the effects of RNAi were relatively limited. Therefore, Raptor/mTORC1 is a potential molecular target to protect against the progression of inflammation-mediated intervertebral disc degeneration.

SIGNIFICANCE: Selective gene silencing of Raptor/mTORC1 could be a new therapeutic strategy for degenerative, inflammatory disc disease.

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ACKNOWLEDGEMENTS: This work was supported by JSPS KAKENHI Grant Numbers 21K09323.

FIGURES: