Cadherin 2 Is Essential To Tie2+ Notochordal Cell Maintenance In Nucleus Pulposus

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Introduction: The function and homeostasis of intervertebral disc (IVD) requires integrity of the nucleus pulposus (NP). Understanding how NP cells survive and being maintained under stress is fundamental to the management of IVD degeneration. Primitive NP consists of a tightly packed notochordal cell (NCC) mass with vacuolated appearance. We have previously illustrated in a mouse model that segregation of this NCC mass is among the earliest events of induced IVD degeneration (1), implying a role of cell-cell adhesion in NCC activities. Cadherins are transmembrane glycoproteins that mediate calcium dependent cell adhesion (2). By transcriptome profiling, we have revealed the expression of Cdh2 gene, encoding cadherin 2/N-cadherin, in rodent NP. Our pilot study has indicated a reduction of cadherin 2 positive cells when the fetal NP becomes mature in human IVD (data unpublished), further supporting the association between cadherin 2 function and NCC maintenance. To date, the role of cadherin 2 in the NP and IVD degeneration remain elusive. We hypothesize that cadherin 2 has a function to regulate the phenotype and survival of the NCC, and that its functional deregulation has adverse impact on IVD homeostasis. We aimed to study the expression pattern of cadherin 2 in IVD during development, aging, and degeneration, and to investigate its function via NP-specific gene and protein ablation strategies. Methods: Under the approval of ethics committee, vertebral columns of wild-type C57BL/6N mice were collected at different ages from embryonic day (E) 15.5 to 2 years old (n=4). To study IVD degeneration, progressive disc degeneration was induced by annulus puncture in 4-month-old inbred Lewis rat tail IVD with 25G needle, and the discs were harvested after 2-, 4-, and 8-weeks of operation (n=6). Immunostaining was performed to study the cadherin 2 expression pattern in the IVD samples. To ablate cadherin 2 protein function, 4-month-old rats were anesthetized and the tail IVDs were surgically exposed for injection of rabbit anti-cadherin 2 antibody or control rabbit IgG (n=6) into the NP via a 34G hypodermic needle. On the other hand, cadherin 2 gene (Cdh2) was knocked out in the NP using a notochord-specific Foxa2-Cre recombination strategy. For both ablation models, disc height was measured and expressed as disc height index (%DHI) to assess IVD degeneration status. The discs were harvested by 2 and 8 weeks after operation (protein ablation) and from P0 and 1 month-old mutants (gene knockout) for histological analysis. Immunostaining was conducted to test the expression of cadherin 2 and others proteins including chondrocyte marker collagen II, vacuole membrane marker Rab32 and disc progenitor marker Tie2.

Results: Expression analysis showed relatively low cadherin 2 signals in mouse embryonic notochord and in newborn NP. However at 3- and 6-month old, strong cadherin 2 signals were specifically detected along the cell-cell junctions of the NCC in the NP (Fig. a). In aged IVD (2-year old), the NCC were replaced by chondrocyte-like cells with lower expression of cadherin 2. In puncture-induced rat disc degeneration, the NP was found to be populated by chondrocyte-like cells showing reduced level of cadherin 2 expression. Functional ablation in vivo by injection of cadherin 2 antibody caused a reduction...
of cadherin 2 expression along with decreased disc height when compared with the IgG injection control (Fig. b). Moreover, the NCC mass showed sign of segregation and attained a chondrocyte-like morphology with upregulation of collagen II expression. Conditional Cdh2-/- mice (CKO) showed absence of cadherin 2 in the NCC and displayed significantly smaller body size and reduced disc height by 1-month old (Fig. c). Notably, the NCC in CKO mice show reduced vacuolated appearance at P0 and a complete loss of vacuolated phenotype with disorganized inner annulus by 1-month of age (Fig. d). The 'vacuole-less' NCC retained the expression of Rab32, but showed a loss of Tie2 expression.

**Discussion:** Our data showed that cadherin 2 is specifically and homogeneously expressed in young primitive NP, indicating that cadherin 2 can mark the NCC population in IVD. The lower expression of cadherin 2 observed in the aged and degenerative discs substantiate its association with IVD degeneration. Conversely, our two independent in vivo models of functional ablation demonstrated a role of cadherin 2 in maintaining NCC phenotype and the structural integrity of IVD. Rab32 is a GTPase expressed in the notochord during vacuole inflation and regulates vacuole formation (3). The loss of vacuolated appearance without change in Rab32 expression suggests that the NCC in the NP undergo atypical vacuole genesis or contain pseudo-vacuoles. Remarkably, Cdh2-/- NCC lost Tie2 expression, which is suggested to label the disc progenitor population (4). Our study therefore illustrates that the maintenance of Tie2+ NCC, which possibly represent a progenitor-like subpopulation, requires a function of cadherin 2.

**Significance:** To our knowledge, this is the first study to demonstrate the essential role of cadherin in NCC maintenance and IVD homeostasis in vivo. Manipulation of cadherin 2 activity may provide a unique approach to delineate the biology and function of NCC in future.