Depleting TGFBR2 In Cartilage Protects Against Spontaneous Hip OA In BALB/c Mice

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INTRODUCTION: Upregulation of integrin α1β1 in the superficial zone of cartilage protects the knee in the early stages of spontaneous and post traumatic OA[1,2,3]. Work in the kidney has shown that integrin α1β1 inhibits TGFBR2 signalling by recruiting the tyrosine phosphatase TCPTP and dephosphorylating tyrosines on TGFBR2[4]. Work in cartilage has shown that depleting TGFBR2 in the cartilage of *itga1*-null mice attenuates the early and worse spontaneous OA seen in the *itga1*-null mouse knee[3]. Together these data suggest that integrin α1β1 protects against knee OA by dampening TGFBR2 signalling at the onset of the disease. It is currently unknown if *itga1*-null mice also develop early and worse spontaneous OA in the hip, and the role of TGFBR2 signalling in the hip. Therefore, the purpose of this study was to evaluate spontaneous hip OA in mice lacking integrin α1β1 (*itga1)* ubiquitously and *tgfbr2* selectively in the cartilage. We hypothesized that *itga1*-null mice would develop early and worse spontaneous OA in the hip relative to wild type mice, and depleting TGFBR2 signalling in the cartilage of *itga1*-null mice would attenuate this disease.

METHODS: All animal procedures were approved by the University of Guelph Animal Care Committee. *Itga1*-null, tamoxifen regulated collagen 2 Cre (Col2CreER^T), and *tgfbr2*$^{flox/flox}$ BALB/c mice were bred together to establish mice with/without ubiquitous *itga1*-null at birth, and with/without a cartilage specific depletion of TGFBR2 following intraperitoneal injection of tamoxifen at 14 and 16 days old[3]. Five female and five male mice per genotype group were sacrificed at 4, 8 and 12 months, and eight male and eight female mice were sacrificed at 16 months[3]. Hips were harvested, fixed, and decalcified for 6 days. Following paraffin embedding, hips were transversely sectioned (8 µm) and stained with hematoxylin, fast green and safranin-O[3]. Five sections at 50-60 µm intervals through the cartilage-cartilage contact region of the hip were graded (OARSI scoring system) by two blinded graders. Summed and maximum scores were calculated for the anterior and posterior, acetabulum and femoral head, of each hip. A multivariate ANOVA was conducted on OARSI scores with independent variables including age (4, 8, 12, 16 months), sex (female/male), genotype (*itga1*-null (a), *tgfbr2*$^{flox/flox}$ (T), Col2CreER^T (C) +/-, injected with tamoxifen (t) or corn oil control (c)) and site (anterior and posterior, acetabulum and femoral head).

RESULTS: Immunohistochemistry confirmed a 70% depletion of TGFBR2 in the cartilage of TC+t and aTC+t mice[3]. Cartilage degeneration was site specific and was influenced by a genotype and age interaction (P<0.035) **[Fig 1]**. Wild type mice with intact TGFBR2 signalling (TC+c and TC-t) developed earlier and worse hip OA when compared to wild type mice with depleted TGFBR2 signalling (TC+t) and all *itga1*-null mice **[Fig 1A&B]**. Spontaneous hip OA was isolated to the posterior acetabulum where vertical clefts and erosions were present in the articular cartilage **[Fig 1B&C]**.

DISCUSSION: We show that BALB/c wild type mice developed spontaneous hip OA at 16 months of age, and that depletion of TGFBR2 signalling in cartilage attenuated this OA. These data provide *in vivo* evidence that TGFBR2 signalling drives spontaneous hip OA in BALB/c mice. In addition, OA was primarily manifest on the posterior acetabulum, the location that is initially loaded upon foot contact with the ground[5]. Surprisingly, *itga1*-null mice with heightened TGFBR2 signalling were protected from spontaneous hip OA, and depletion of TGFBR2 signalling in the cartilage of these mice had no effect on OA. This contrasts with the early development of spontaneous OA relative to controls reported in the *itga1*-null knee which is attenuated with TGFBR2 depletion in cartilage[3]. It is important to consider these data in light of the fact that TGF-β is only activated when it is released from the latency associated protein primarily by shear stress. It is conceivable that the cartilage and synovial fluid of the ball and socket hip joint would experience higher magnitude shear stresses compared to the hinge-like knee joint, and thus have inherently higher levels of active TGF-β and TGFBR2 signalling even in wild type mice. Interestingly, the Col6a1-null mouse manifests a similar dichotomy of OA between hip and knee, however in that mouse model OA is accelerated in the hip and protected in the knee[6,7].

SIGNIFICANCE/CLINICAL RELEVANCE: The role of TGFBR2 in spontaneous OA differs between the hip and knee. Molecular treatments of spontaneous knee OA may not translate to the hip.

REFERENCES: **[1]** Zemmyo et al *Arthritis Rheum* 48:10 2003; **[2]** Shin et al *OA Cart* 24:10 2016; **[3]** St Amant et al *OACO* 5:4 2023; **[4]** Chen et al *J Clin Invest* 124:8 2014; **[5]** Li et al *J Biomech* 115:110163 2021; **[6]** Alexopoulos et al *Arth Rheum* 60:3 2009; **[7]** Christensen et al *PLoS One* 7:3 2012.

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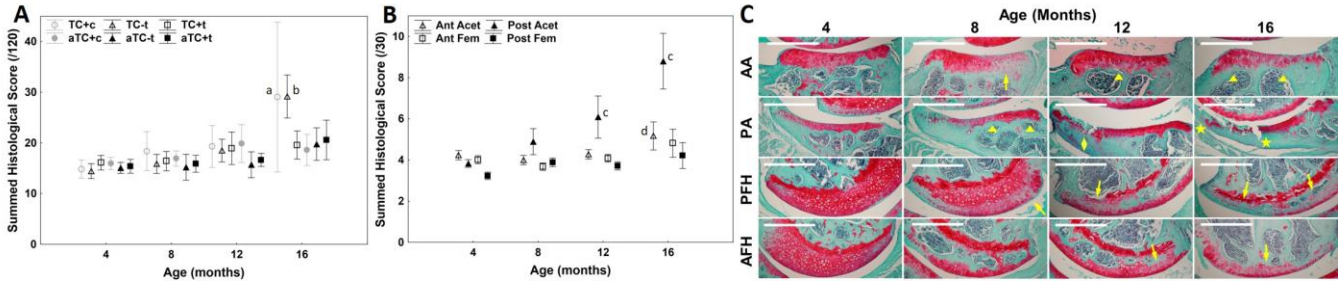

FIGURE 1: Summed OARSI histological scores as a function of (**A**) genotype *(itga1*-null (a), TGFBR2$^{flox/flox}$ (T), Col2CreER^T (C) +/-, injected with tamoxifen (t) or corn oil control (c)) or (**B**) site (anterior/posterior acetabulum/femoral head), and age (months). Points are means ± 95% CI. Note increasing scores with age and greater scores at 16 months for (**A**) TC+c and TC-t mice, or (**B**) the posterior acetabulum. **a,b** significantly different from all other points except for TC-t or TC+c equivalent respectively (P<0.003) **c** different from all other data points (P<0.032) **d** different from all other data points except posterior acetabulum at 8 months and anterior femoral head at 16 months (P<0.036) (**C**) Transverse histological sections from the posterior acetabulum (PA), posterior femoral head (PFH), anterior acetabulum (AA) and anterior femoral head (AFH) in the hips of male mice at 4, 8, 12, or 16 months of age. Sections stained with hematoxylin (nuclei black), fast green (collagen blue), and safranin-O (proteoglycans pink). Scale bar 500 µm. Note proteoglycan loss (arrow), surface fibrillations (triangle), vertical clefts (diamond), and erosion (star).