**A1** A NOVEL SMALL THIENOINDAZOLE-DERIVATIVE COMPOUND PROMOTES CHONDROGENIC DIFFERENTIATION WITHOUT INDUCING HYPERTROPHY THROUGH PRODUCTION OF RUNX1

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Purpose: Aiming at regeneration of permanent cartilage like joint cartilage, this study screened natural and synthetic compound libraries to discover a novel compound inducing chondrogenic differentiation without hypertrophy. We further investigated the underlying molecular mechanism.

Methods: As an efficient monitoring system for chondrogenic differentiation, we established stable lines of mouse chondrogenic ATDC5 cells expressing green fluorescent protein under the control of type II collagen promoter fused with four repeats of a Sox9 enhancer (COL2-GFP). Chondrogenic differentiation was assessed by real-time RT-PCR for COL2, aggrecan, chondromodulin-1, and COL10, toluidine blue and Alcian blue stainings, and quantitative GAG assay in cultures of mouse embryonic stem cells or immature mesenchymal C3H10T1/2 cells. The downstream molecules were screened by a microarray analysis using C3H10T1/2 cells. The COL2 promoter activity was determined using HuH-7 cells transfected with a luciferase-reporter gene construct containing the COL2 promoter above, and the specific binding to the identified region was verified by electrophoretic mobility shift assay (EMSA) and chromatin immunoprecipitation (ChIP). For functional analyses, we performed adenoviral overexpression of the gene or the small interfering RNA in C3H10T1/2 cells, and compared the chondrogenic differentiation as described above with respective empty-vector controls. Molecular interactions were examined by immunoprecipitation, mammalian two-hybrid system, and immunohistochemistry in the mouse growth plate. Finally, cartilage formation in full-thickness defects of mouse knee cartilage was histologically evaluated after transplantation of cell-sheets of mouse rib chondrocytes with or without TM treatment.

Results: The COL2-GFP system showed that a small thienoindazole-derivative compound T-198946 (TM) most strongly induced the GFP fluorescence as early as after 48 h of treatment. TM was confirmed to enhance chondrogenic differentiation but inhibit the further hypertrophic differentiation in the cultures of precursor cells. The microarray screening revealed that Runx1 was most strongly induced by TM among 581 up-regulated genes including Sox5 and Sox6. Deletion, mutagenesis, and tandem-repeat analyses of the luciferase assay identified the core responsive element of Runx1 in the COL2 promoter to be between the −293 and −288 bp region containing a putative Runx-binding motif. EMSA and CHIP assays confirmed the specific binding of Runx1 to this region. Although chondrogenic differentiation of C3H10T1/2 cells was little enhanced by the Runx1 overexpression alone, it was much enhanced by co-transfection with Sox5, 6, and 9 (the Sox trio), without inducing the hypertrophy, as true of the effect of TM treatment. Gene-silencing of Runx1, Sox5/6, or Sox9 suppressed the TM effect on chondrogenic differentiation. In fact, Runx1 and the Sox trio were co-localized in the proliferative and pre-hypertrophic cartilaginous tissue and their interaction was confirmed by immunoprecipitation and two-hybrid analysis. Finally, cell-sheets of TM-treated chondrocytes filled the defects with cartilaginous tissue, while the control cell-sheets did not.

Conclusions: A novel small compound TM promotes chondrogenic differentiation without inducing hypertrophy, through production of Runx1 that cooperatively functions with the Sox trio. TM will herald a new era of regenerative medicine of permanent cartilage, thus realizing an epochal treatment of osteoarthritis.

**A2** IMPACT OF GENDER ON QUALITY-ADJUSTED LIFE EXPECTANCY LOSSES DUE TO KNEE OSTEOARTHRITIS IN THE US ELDERLY POPULATION

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Purpose: The burden of osteoarthritis (OA) is best measured by its impact on quality of life. Women are disproportionately affected by knee OA. Our objective was to estimate the quality-adjusted life expectancy losses for elderly women and men with symptomatic knee OA in the US.

Methods: We used NHANES III data to estimate the proportion of men and women >60 years of age who had symptomatic knee OA. We applied this distribution to the total population of persons >60 years of age (obtained from 2000 US Census data) to estimate the actual number of elderly persons with symptomatic knee OA in the US, stratified by sex. Person survival losses adjusted for quality of life were estimated using the Osteoarthritis Policy Model (OAPol), a comprehensive computer simulation model of natural history and clinical management of knee OA. Model input parameters included data on the comorbidities and quality of life decrements associated with symptomatic knee OA, obesity (BMI ≥ 30) and other chronic diseases derived from NHANES III. OA progression rates were estimated from the Johnston County Osteoarthritis Project and calibrated using published literature. Background mortality rates (age, sex, race-adjusted) were derived from US life tables.

Results: Approximately 10% (8.3 million) Americans (≥60 y/o) have symptomatic knee OA, 64% of whom are women. Women with symptomatic knee OA will experience loss of approximately 6.3 million of quality-adjusted years compared to 3.5 million quality-adjusted years of life lost for men. Obesity, resulting in 65% of such losses, is a substantial contributing factor (Figure) for both men and women.

Conclusions: There is a disproportionate burden of knee OA in women, particularly in those women who are obese. These substantial losses in quality-adjusted life expectancy underscore the importance of including quality of life in assessment of public health and clinical interventions focused on reducing the burden of knee OA. More research is needed to understand the relative contributions of biology and environmental exposures in explaining the differences observed between men and women in OA-related disability.