Whole-genome sequencing of human chondrocytes reveals genome instability due to endogenous mutagenic factors

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INTRODUCTION: Human somatic cells are continuously exposed to various DNA damaging agents that can cause mutagenic lesions and result in mutations and genome instability. Somatic mutations and rearrangements are associated with cellular aging and diseases. Aging is the primary risk factor for developing osteoarthritis (OA). Previous studies using comet assay have shown high DNA damage burden in chondrocytes from older donors [1]; however, our understanding of genome dynamics during chondrocyte aging is lacking. Here, we take a whole-genome sequencing approach to evaluate the load of genome changes in chondrocytes from donors with and without OA. We also compare these data with those produced by the same methodology in human skin fibroblasts [2] as an initial investigation into whether chondrocytes, as a cell type that rarely undergoes mitosis after skeletal maturity, has a distinct repertoire of genome changes as compared to a mesenchymal cell type with a higher division rate.

METHODS: Skin fibroblasts were harvested from cells that grew out of punch biopsies of the forearm (sun-exposed) and hip (non-exposed) of donors 25 to 79 years old as described [2]. Chondrocytes were isolated from either end-stage OA (surgical waste tissue at total knee replacement) or from healthy femur cartilage (cadaveric donors with macroscopically normal cartilage and no clinical history of joint disease). Allowable use of tissue was confirmed by institutional IRBs. Chondrocytes were maintained in monolayer culture before generation of single-cell derived colonies that expand to over one million cells [3]. DNA was isolated from two independent colonies and a bulk sample for each of 6 OA (3 males aged 60, 69, and 86; 3 females aged 55, 62, and 73) and 3 healthy donors (1 male aged 73, 2 females aged 56 and 59). DNA library was prepared with Watchmaker DNA Library Prep Kit with Fragmentation. All samples were sequenced at 34X to 114X depth on either NovaSeq 6000 or DNBSEQ-T7 sequencers. FASTQ files were aligned to the hg19 genome using BWA-MEM. Mutation calls were generated by consensus of VarScan2 and Strelka2 with bulk chondrocytes of the same donor used as a matched normal. To eliminate mutations acquired during culture expansion, mutations were filtered to retain heterozygous mutations with allele frequency 45%-55%, or homozygous mutations with allele frequency ≥90%. Patterns of somatic mutations and associated mutagenic processes were identified by motif-centered analysis using P-MACD package. Delly was used to identify structural variants in the form of deletions, duplications, insertions, inversions, and translocations.

RESULTS SECTION: For chondrocytes, we detected 113 to 624 base substitutions per donor, with C→T changes being the most common (Fig A). There was no statistically significant difference between chondrocytes from age-matched donors with or without OA. The total mutation burden in chondrocytes was 4.5-fold and 14.5-fold lower compared to non-exposed and sun-exposed fibroblasts, respectively. Analysis of mutational motifs identified two motifs that were present in both chondrocytes and fibroblasts: aTn→aCn is associated with small epoxides and S_N2 electrophiles, whereas nCg→nTg is caused by spontaneous deamination of meCpG motifs. The UV-associated motif yCn→yTn was only detected in fibroblasts (Fig B). The donor average aTn→aCn motif minimum estimate of mutation load (MEML) ranged from 6 to 28 in chondrocytes and 0 to 31 in fibroblasts. nCg motif MEML ranged from 10 to 82 in chondrocytes and 27 to 98 in fibroblasts. yCn motif MEML ranged from 0 to 2,640 in fibroblasts. The average number of structural variants detected for individual donors ranged from 1 to 9 in chondrocytes and 1 to 13 in fibroblasts, with deletions contributing to the largest fraction of rearrangements detected (Fig C).

DISCUSSION: Declining DNA repair efficiency and damage accumulation have been proposed to expedite chondrocyte senescence and OA progression. Unrepaired DNA lesions lead to somatic mutations and gross chromosomal rearrangements. In addition to the possibility that these alterations directly affect cellular function, understanding the types of mutagenic changes provides insight into the extent and mechanisms of DNA damage in cartilage tissue. This study measures the baseline somatic genome changes in chondrocyte genomes using whole-genome sequencing of single-cell clonal lineages and compares findings to single-cell clonal lineages of human dermal fibroblasts. We detected a higher burden of mutations in fibroblasts compared to chondrocytes, indicative of the unique exposure to DNA damaging agents in different tissues of the human body. Mutation loads associated with small epoxides (aTn→aCn) and deamination of meCpG (nCg→nTg) were comparable between chondrocytes and fibroblasts despite the significantly lower total number of mutations in chondrocytes. This alludes to the endogenous and universally operational nature of these two mutagenic processes across the human body and contrasts with the activity of environmental mutagenic agents operational only in the exposed tissues, as exemplified by the detection of UV-associated motif yCn→yTn in fibroblasts but not in chondrocytes. We did not detect any difference between healthy and OA chondrocytes for mutation load, mutational motif load, or the number of rearrangements. This was a surprising finding given that OA chondrocytes have higher levels of overall DNA damage. One possible explanation is that the types of DNA damage that occur during OA have low potential for conversion to mutagenic lesions. Another explanation is that OA chondrocytes with high mutational load are selectively depleted by the time of isolation at end-stage disease or during in vitro expansion. Of note, a recent study using single-cell multiple displacement amplification found that OA chondrocytes had a lower mutational burden as compared to non-OA chondrocytes [4].

SIGNIFICANCE/CLINICAL RELEVANCE: Our study investigated alterations across the whole genome for chondrocytes from healthy and OA donors. Utilizing motif analysis provides insight into the sources of mutagenic DNA damage, which can be used to develop strategies for counteracting DNA damage and senescence as a possible therapy for OA.

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