Telomerase activity is lost in articular chondrocytes after puberty.  

INTRODUCTION

Increased age is the most significant predisposing factor for the development of osteoarthritis (OA). As age increases, cells reach replicative senescence, the maximum number of times a somatic cell can divide. It has been suggested in previous studies that replicative senescence is initiated by telomere erosion, which occurs after the chromosomal telomere falls below a critical length. Telomeres, located on the end of chromosomes, protect chromosome ends from undergoing degradation and maintain chromosome stability during replication. In order to retard the natural shortening of telomere length, telomerase, a ribonucleoprotein, adds telomeres to the ends of eukaryotic chromosomes. A decline in telomerase activity during the ageing process is linked with telomere shortening.

Because ageing and development of OA are highly correlated, we wanted to examine the potential role of telomerase in OA onset. Chondrocytes are key in preserving cartilage health, and previous studies have shown that chondrocyte responsiveness to external stimuli changes with increased age, implying a general loss in cellular homeostasis with age. Subsequently, chondrocytic telomerase activity may become altered with increased age.

Our hypothesis for this study was that telomerase activity in articular chondrocytes would decrease with increasing age, declining at a specific, physical maturity level, which most likely will correspond with puberty.

METHODS

Cell culture. Cartilage was harvested from articular joints of horses (ages 0 to >24 mos) and chondrocytes were isolated using collagenase. Chondrocytes were grown in monolayer in Ham’s F-12 + 10% FBS media for 24 hours, then trypsinized, counted (2 x 10^6) and lysed. Telomerase activity. Telomerase activity was determined using the TeloTAGGG Telomerase PCR ELISA kit (Roche Applied Science, Penzberg, Germany). The telomerase detection kit is composed of two steps, a Telemetric Repeat Amplification Protocol (TRAP) reaction, and an Enzyme-Linked Immunosorbent Assay (ELISA) step, both of which were performed according to the protocol provided in the kit. The telomerase activity was determined in four age groups: pre-pubescent (0-7.5 months), pubescent (7.5-15 months), post-pubescent (15-24 months), and mature (>24 months) as defined previously. 1 According to the manufacturer’s directions, samples were considered telomerase-positive if the difference in absorbance between the sample and the negative control was greater than 0.2 A450 nm – A690 nm units.

Statistical analyses. Statistical analyses was performed using a Fisher’s exact test to assess the categorical data (i.e. A450 nm – A690 nm ≥0.2 = yes; A450 nm – A690 nm <0.2 = no). Telomerase positive groups were compared to telomerase negative groups.

RESULTS

Telomerase activity was present in pre-pubescent and pubescent horses and absent in both age groups of horses beyond pubescence. The average telomerase activity in chondrocytes from the pre-pubescent and pubescent horses exceeded the A450 nm – A690 nm was 0.3738 in pre-pubescent and 0.3554 in pubescent groups. In post-pubescent and mature chondrocytes, the A450 nm – A690 nm was 0.07332 and 0.05577, respectively; therefore the samples did not have detectable telomerase activity (Figure 1). Telomerase activity in prepubescent and pubescent horses was significantly greater than telomerase activity in post-pubescent and mature horses (p = 0.0076). Pre-pubescent animals were not significantly different from pubescent animals (p = 0.50), and similarly, post-pubescent samples were not significantly different than mature (p = 0.79).

DISCUSSION

Most previous studies have focused on telomerase activity in immortalized cells or cancerous cells; however, this study examined primary cells isolated from somatic tissue of young (pre-pubescent) animals through mature aged animals. Our results indicate that telomerase activity is present in articular chondrocytes until the horse enters the post-pubescent stage at approximately 15 months of age, as defined by serum insulin-like growth factor-I and insulin-like growth factor-I binding protein concentrations and full formation of the tidemark in articular cartilage. 1 Other studies have similarly shown a decrease in telomere length related to chondrocyte ageing in vitro. 2 The time frame observed in this study Our results are also consistent with studies that demonstrate diminished responsiveness of aged chondrocytes to anabolic stimulation with insulin-like growth factor-I, and implicates that these changes might begin as early as puberty. These data suggest that aging of cartilage begins as early as post-puberty and that investigations into therapeutics to enhance cartilage repair should carefully consider the age of target populations in laboratory and clinical studies. The loss of telomerase and the termination of replication due to an absence of telomerase post-pubescence may play a role in the limited capacity for articular cartilage repair in adults.

REFERENCES