The role of extracellular vesicles in synovial fibroblast senescence

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Introduction

Senescent cells accumulate in ageing tissues ageing and age-related accumulation of senescent cells promotes ageing. They produce reactive oxygen species (ROS), have dysregulated mitochondrial function and metabolism, limited tissue regeneration and secrete bioactive molecules, including pro-inflammatory cytokines, chemokines and matrix-remodelling enzymes (senescence-associated secretory phenotype). *In vitro* senescent cells induce a senescent phenotype in surrounding bystander cells via ROS. Extracellular vesicles (EVs) are mediators in intercellular communication with critical roles in cellular senescence and ageing. In arthritis synovial fibroblast (SF) senescence is linked to the activation of a pro-inflammatory phenotype contributing to chronic arthritis pathogenesis. We hypothesise that senescent cells in osteoarthritic SFs induce senescence and/or a pro-inflammatory phenotype in non-senescent osteoarthritic SFs, mediated through EV cargo.

Materials and Methods

SFs were cultured from synovial tissue biopsies collected from osteoarthritis patients (n=3) undergoing elective joint replacement (NRES 16/SS/0172). Small RNAseq was undertaken on EVs isolated from the secretome of non-senescent and from the secretome of irradiation-induced senescent SFs (n=3) and differentially expressed sncRNAs identified using size exclusion chromatography, and characterised by nanoparticle tracking and electron microscopy. Senescence was confirmed by beta-galactosidase staining and microscopy. The inflammatory phenotype was assessed using an interleukin-6 ELISA. Bioinformatics was conducted in Ingenuity Pathway Analysis (IPA).

Results

Beta-galactosidase staining confirmed senescence. Irradiation induced senescence had no significant effect on interleukin-6. A diverse EV RNA content was evident including snoRNAs, snRNAs, tRNAs, lncRNA, y-RNA, scRNA, mRNAs, rRNAs and microRNAs, with tRNAs being the most abundant (11.5% total reads). We found 11 lncRNAs, 13 tRNAs and 1 snRNA differentially expressed. We inputted 18 of the most differentially expressed microRNAs into IPA and identified fibrosis (p=2.5E-6), cell proliferation (p=6.8E-6), autophagy (p=0.001) and cell cycle (p=0.001) as significant pathways.

Discussion

Understanding the exact composition of EV-derived sncRNA of senescent cells will increase our knowledge about their role in intercellular communication and potential role as active molecules in the senescence bystander effect. Interestingly, our literature search revealed half of the 17 most regulated microRNAs had previously been identified as having roles in either senescence or inflammation. Furthermore, it has been proposed that different types of senescence could lead to the expression of different pools of tRNAs.