APOPTOSIS OF SYNOVIAL FIBROBLASTS INDUCED BY P53-DERIVED HYBRID PEPTIDES THROUGH DISOCIATING THE BINDING OF P73 WITH IASPP TO INCREASE PUMA AND BAX EXPRESSION

C.-R. Wang1, S.-Y. Chen2, A.-L. Shiau2, C.-L. Wu3. 1. Internal Medicine, National Cheng Kung University Hospital, 2. Microbiology and Immunology, 3. Biochemistry and Molecular Biology, National Cheng Kung University Medical College, Tainan, Taiwan.

Background: In rheumatoid arthritis (RA) synovial fibroblasts (SFs), mutant p53 can lead to transformation-like features resistant to the apoptosis induction. Deficiency in p53-mediated suppression by its dominant-negative counterpart is observed in human cancers with activating p73 as a therapeutic strategy in such patients. A p53-derived hybrid peptide 37 amino acid (37AA) can inhibit the p73 binding with inhibitory apoptosis stimulating protein of p53 (IASPP), thus activating the downstream apoptosis signaling pathway in tumour cells.

Objectives: We hypothesised that p73 is involved in the RA pathogenesis, and examined whether p53-derived hybrid peptides can activate p73 to induce apoptosis of SFs by using adenosine vectors encoding 37AA (Ad37AA) to transduce SFs in vitro and inject collagen-induced arthritis (CIA) joints in vivo.

Methods: Mononuclear cells (MNCs) from RA patients before and after receiving the adalimumab therapy were examined for IASPP expression by real-time RT-PCR. Synovial tissues and SFs from RA patients and CIA rats were subjected to immunohistochemical and immunofluorescent staining and real-time RT-PCR for the expression of IASPP, PUMA and Bax. SFs were transfected with lentiviral vectors-encoding short hairpin p73 RNA to produce p73-silenced SFs. P53-derived hybrid peptides were added to the culture media, and TUNEL assay for apoptotic status and real-time RT-PCR for the expression of IASPP, PUMA and Bax were performed.

Results: There were reduced IASPP levels at 48 hours post adenovirus (Ad37AA) infection in RA patients. IASPP expression was reduced in Ad37AA-infected SFs, and silenced p73 abrogated the increased PUMA and Bax expression. Articular indexes and histologic scores were reduced in Ad37AA-injected joints with decreased SF densities, increased apoptotic cells, higher PUMA expression and P53 expression.

Conclusions: These results demonstrate that injecting p53-derived hybrid peptides can induce apoptosis of SFs through the activation of p73 in the rheumatoid joint, suggesting that the p73-dependent apoptotic mechanism is a potential therapeutic strategy in RA patients.

REFERENCES:

ACKNOWLEDGEMENTS: We thank Dr. Kevin M. Ryan (Cancer Research UK Beatson Institute, Glasgow, UK) for providing pSuttleCMV-37AA adenosial plaques, and Drs, Ming-Fei Liu and I-Ming Jou (National Cheng Kung University Hospital) for providing synovial specimens from arthritis patients.

Disclosure of Interest: None declared
DOI: 10.1136/annrheumdis-2018-eular.1900

IAA ANTI-CCP ANTIBODIES ARE DETECTABLE IN THE SALIVA BUT NOT SPUTUM OF INDIVIDUALS AT- RISK OF DEVELOPING RHEUMATOID ARTHRITIS

K. Markevi1, P. Pentony1, L. Hunt2, Y. El-Sherbiny1, L. Duquenne3, D. Consaidd1, T. Do1, J. Meade1, D. Devine1, P. Emery1. 1. Rheumatology, Leeds Institute of Rheumatic and Musculoskeletal Medicine and NIHR Leeds Biomedical Research Centre, 2. Rheumatology, Leeds Institute of Rheumatic and Musculoskeletal Medicine, 3. Oral Microbiology, University of Leeds, Leeds, UK

Background: Recent evidence suggests the initiation of rheumatoid arthritis (RA) – related autoimmunity may occur by local citrullination at the oral mucosa and lungs. IgA antibodies are the hallmark of mucosal immunity; the majority of saliva IgA antibodies are locally produced whereas IgG antibodies are largely serum derived. Furthermore, IgA anti-CCP antibodies have recently been described in the sputum of at-risk individuals. The relative importance of the oral and lung mucosa in disease initiation is, however, unclear and the prevalence of saliva and sputum anti-CCP antibodies in the same at-risk individuals has not been reported.

Objectives: To investigate the prevalence of IgA anti-CCP antibodies in the saliva and sputum of seropositive individuals at risk of developing RA.

Methods: Anti-CCP positive individuals with no evidence of clinical synovitis (CCP+), anti-CCP positive RA (CCP+RA) and healthy controls (HC) matched for age and smoking status were recruited. Unstimulated saliva and serum samples were collected. Induced sputum samples were obtained using 7% saline via ultrasonic nebuliser (UltraNeb 3000 DA, Devilbiss, Germany). Sputum was mixed with phosphate buffered saline, mechanically disrupted and centrifuged to obtain supernatant. IgA and IgG anti-CCP antibodies (anti-CCP2, immunocap assay, Phadia) were measured in all saliva, sputum and serum samples. IgA and saliva sputum IgG anti-CCP titres exceeding the 95th centile in HC were considered positive.

Results: 55 CCP+; 40 RA and 32 HC were recruited and had saliva and serum collected. 24 CCP+; 14 RA and 22 HC had sputum and serum collected. Of these, 23 CCP+ and 7 RA patients provided simultaneous saliva, sputum and serum samples. 8/55 (15%) CCP+ and 10/40 (25%) RA patients had positive saliva IgA anti-CCP levels compared with 1/31 (3%) HC. 23/54 (43%) CCP+ and 21/48 (44%) RA patients had positive serum IgA anti-CCP levels compared with 1/32 (3%) HC (table 1). None of, 7/18 (39%) patients with a positive saliva IgA anti-CCP test had a negative serum IgA anti-CCP test, suggesting localised production and accumulation of IgA anti-CCP antibodies rather than transfer from the serum. Only 1/24 CCP+ (4%) and 1/14 (7%) RA patients had positive sputum IgA anti-