ANGIOTENSIN II TYPE 2 RECEPTOR (AT2R) IS OVER-EXPRESSED IN SYNOVIAL OA AND RA TISSUE AND INCREASES STEADILY WITH INFLAMMATORY STIMULI: A POTENTIAL NEW TARGET FOR PAIN AND ANTI-INFLAMMATORY THERAPIES

R. TERENZI[1], I. ROSA[2], M. MANETTI[2], F. GALLUCCIO[1], S. GUIDUCCI[1], L. IBBA-MANNESCHI[2], M. MATUCCI CERINIC[1]

[1]S.O.D. Reumatologia A.O.U. Careggi, Università degli Studi di Firenze; [2]Dipartimento di Anatomia Umana, Università degli Studi di Firenze

Scopo del lavoro. Activation of transient potential type I vanilloid receptor (TRPV1) is crucial for pain perception and inflammation. During rheumatoid arthritis (RA) direct stimulation of TRPV1 positive fibroblast-like synoviocites (FLS) promotes secretion of inflammatory citokynes, like IL-6 and IL-8. Despite these data there aren't TRPV1 antagonist available for human therapies. Recently, a new membrane receptor, the angiotensin II type 2 receptor (AT2R) has been discovered as crucial activator of TRPV1.

To demonstrate the presenceof AT2R in synovial tissues of RA and OA patients.

To evaluate expression of AT2R inFLS, macrophages,T and B cellsof RA tissue.

To evaluate differences of expression of AT2R in cultured healthy FLS (H-FLS), osteoarthritis FLS (OA-FLS), RA-FLS at baseline and after IL1β or TNF-α incubation.

Materiali e Metodi. Synovial biopsies were collected from 8 patients with active knee-RA and 8 withOA-knee. Also H-FLS were obtained. For

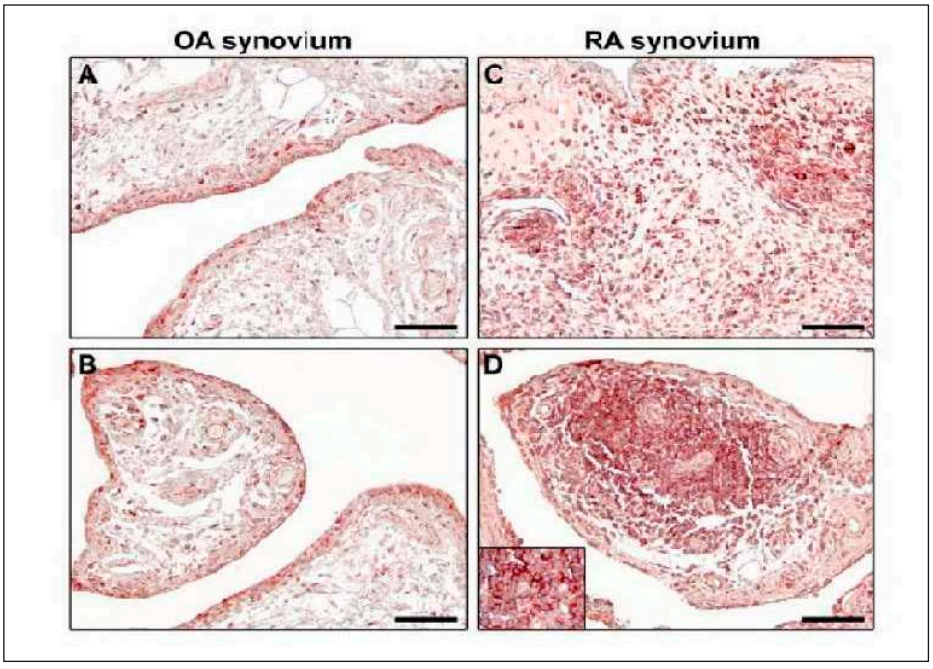

Figure 1

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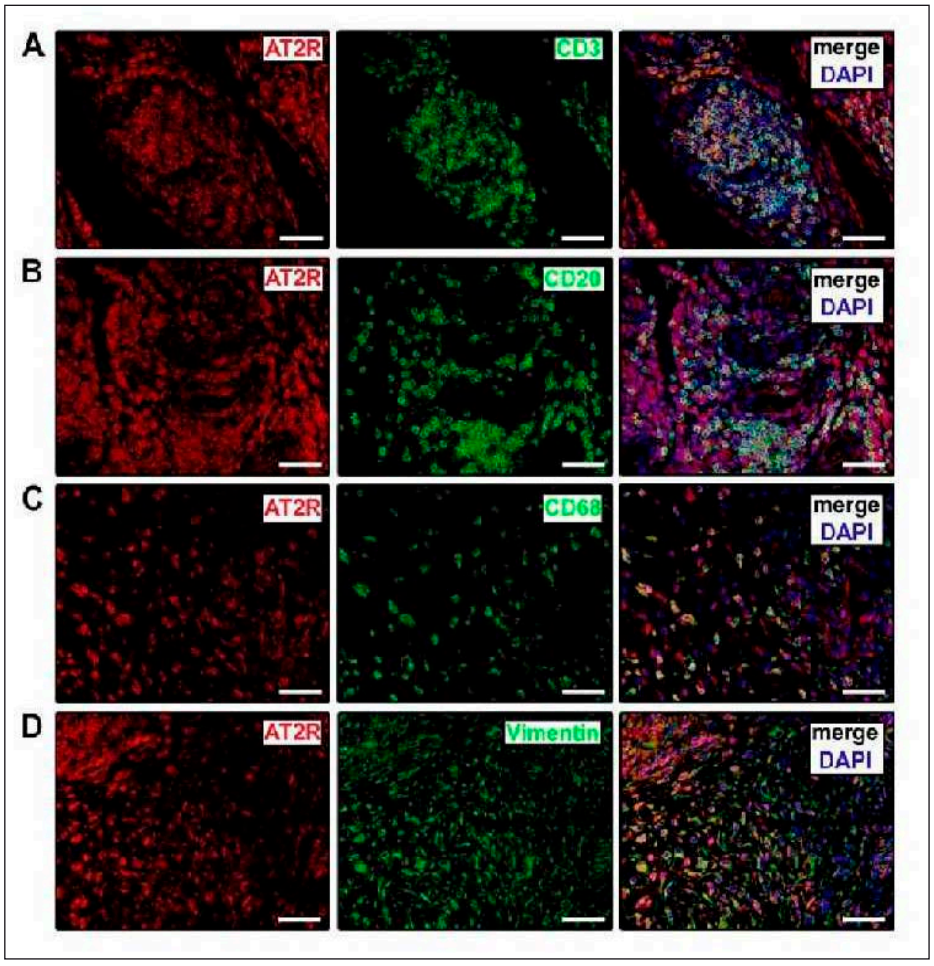

Figure 2

immunohistochemistry, immunocytochemistry and western blotting analysis AT2R antibody were used. For fluorescence immunohistochemistry analysis specimens were also incubated with anti-CD3, anti-CD20, anti-CD68 and anti-vimentin.

Nuclei were counterstained with 4',6-diamidino-2-phenylindole (DAPI). For fluorescence immunocytochemistry and western blotting analysis FLS were serum-starved for 24 hours or incubated with IL1β, TNF-α alone or in combination. For statistical analysis the Student's t-test was used (p value < 0.05 was considered statistically significant).

Risultati. AT2R was express from everylayers of OA and RA synovial samples (Fig. 1, A-D). RA lining layer had significantly higher levels of AT2R than OA (p=0,035) (Fig. 1, A, C, E). Furthermore expression of AT2R was significantly higher in RA sub-lining layer than OA lining layer (p=0,002) (Fig. 1, B, D, E). Merge immunohistochemistry of RA samples showed that FLS, macrophage and T and B cells strongly expressed AT2R (Fig. 2, A-D).

Immunocytochemistry and western blotting analysis showed that quantitative AT2R baseline expression was significantly higher in OA-FLS than H-FLS (p<0,001), nevertheless RA-FLS showed the higher levels of baseline AT2R (p<0,001) (Fig. 3). Incubation with IL1β and TNF-α significantly increased expression of AT2R-FLS baseline levels in all cellular lines (p<0,05) (Fig. 3).

Conclusioni. This study demonstrates, for the first time, the presence of AT2R in human synovial specimens. AT2R was found in every RA and OA samples suggesting a possible constitutive role in RA and OA pathogenesis. HoweverRA samples had higher AT2R expression than OA, both in lining and sub-lining layers. Macrophages, FLS, T and B lymphocytes, leading cells

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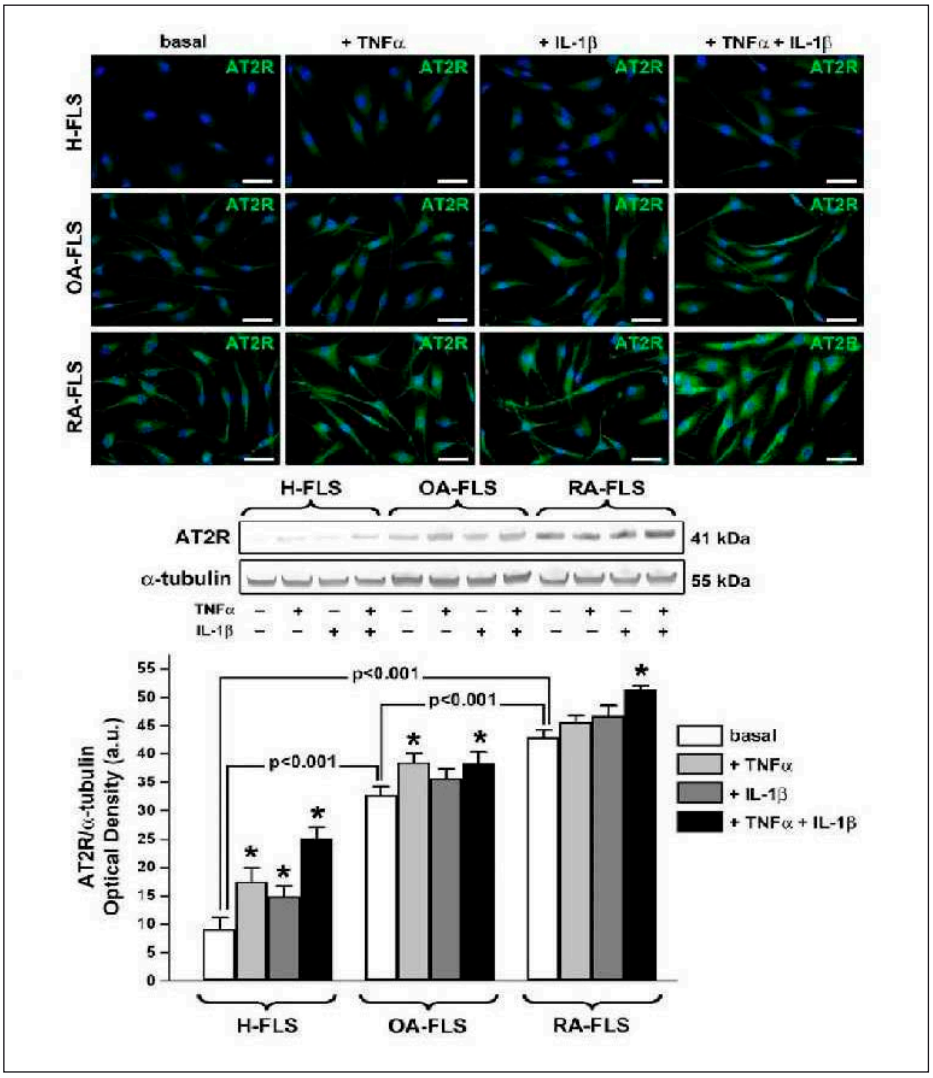

Figure 3

in inducing and sustaining RA inflammation, strongly expressed AT2R. Moreover, inflammatory stimuli induce AT2R expression, as demonstrated by levels of AT2R in H-FLS, OA-FLS and RA-FLS at baseline and after incubation with IL1β and TNF-α. These evidences suggest that AT2R could act as pro-inflammatory receptor in OA and RA. AT2R antagonism could be a potentially new target for OA and RA treatment.

Keywords: *Angiotensin II type 2 receptor, rheumatoid arthritis, new drugs.*

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