Evaluation of Expression of AT1 and AT2 Receptors in Nuclear Membrane of Mesangial Cells

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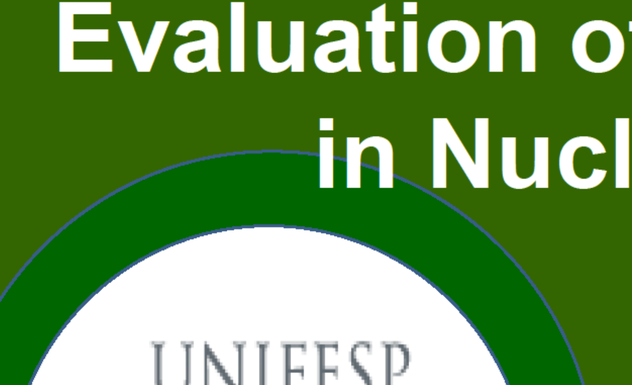
Introduction



Rajesh Kumar, Vivek P. Singh, Kenneth M. Baker, The intracellular renin–angiotensin system: a new paradigm, Trends in Endocrinology & Metabolism, Volume 18, Issue 5, July 2007.

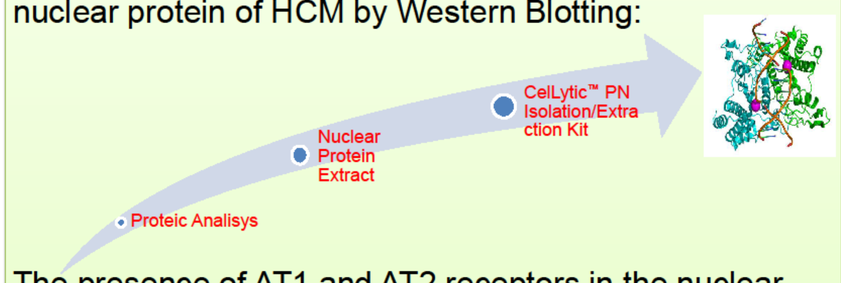
Currently is discussed the biological relevance of the intracellular renin-angiotensin system (RAS). The presence of receptors in the intracellular compartment may mediate nonclassical effects of Ang II, such as, growth, proliferation, and regulation of gene expression of AngII target genes such as the pro-inflammatory and pro-fibrotic.

Objective

The objectives of this study were to evaluate the presence and the ability of AngII to bind to these receptors present in the nuclear membrane of human mesangial cells (HMC).

Methods

Protein expression of AT1 and AT2 was analyzed in nuclear protein of HCM by Western Blotting:



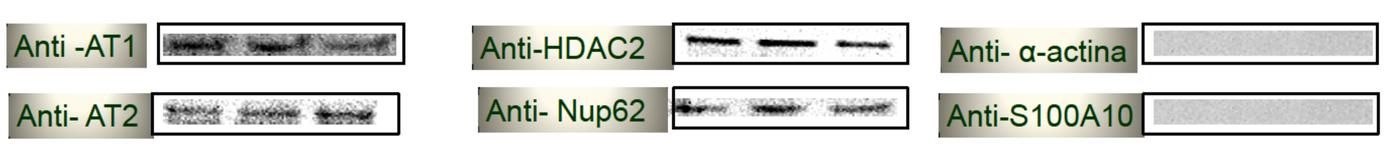
The presence of AT1 and AT2 receptors in the nuclear membrane of the HMC was evaluated in isolated nuclei by immunofluorescence under following conditions:

- ➢Control: Isolated nucleus HMC incubated with secondary antibody conjugated with fluorochrome;
- ➢Isolated nuclei labeled with anti-AT1 and anti-AT2 antibodies;
- ➢HMC pretreated with losartan (AT1 blocker) and PD-123319 (AT2 blocker);
- ➢Isolated nuclei exposed to AngII labeled with fluorochrome-conjugated FITC.

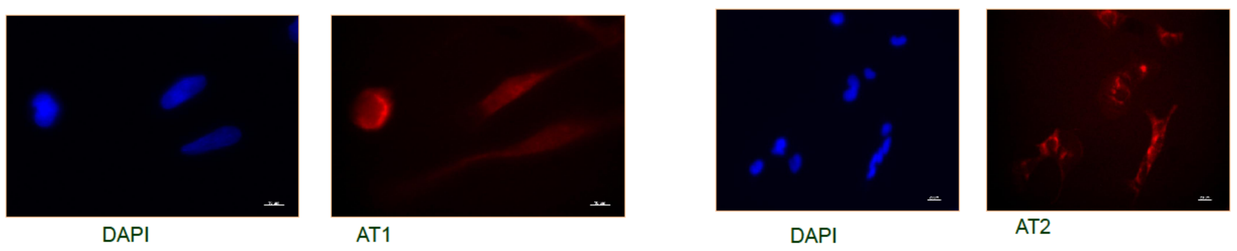
Results

Western Blotting

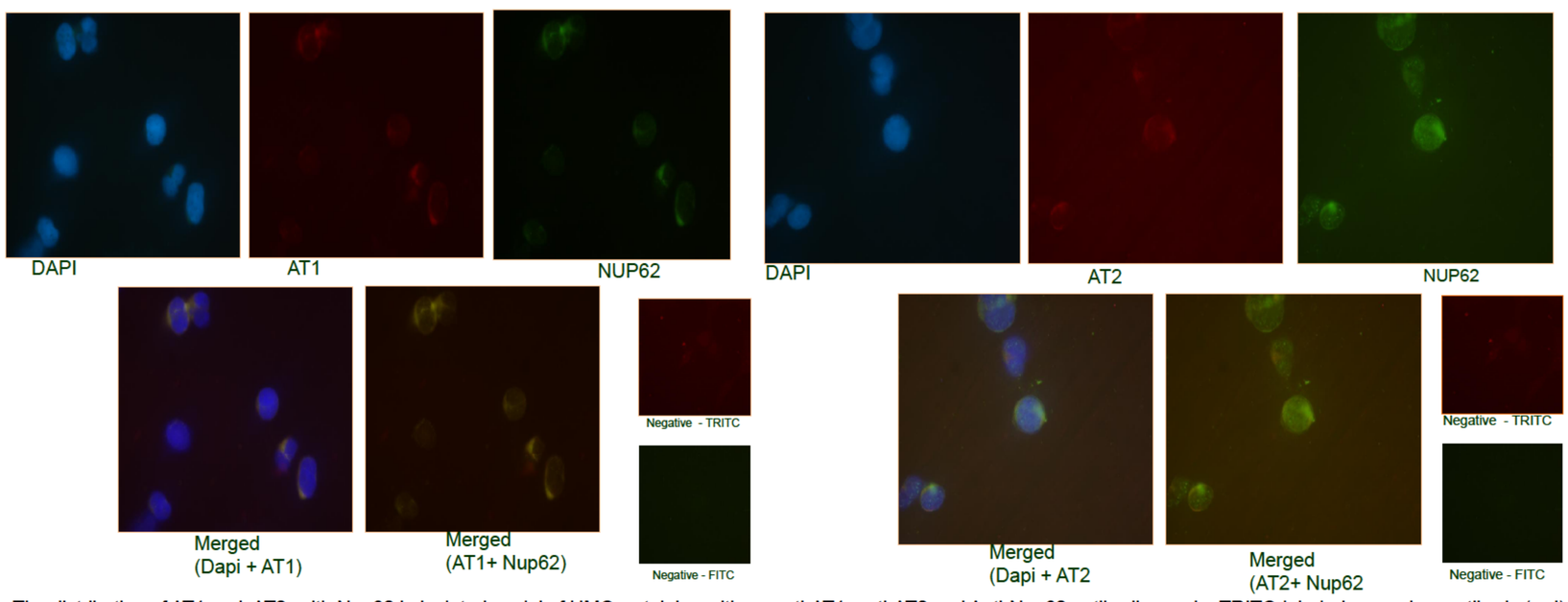
Protein expression of AT1 and AT2 in nuclear protein extract of mesangial cells



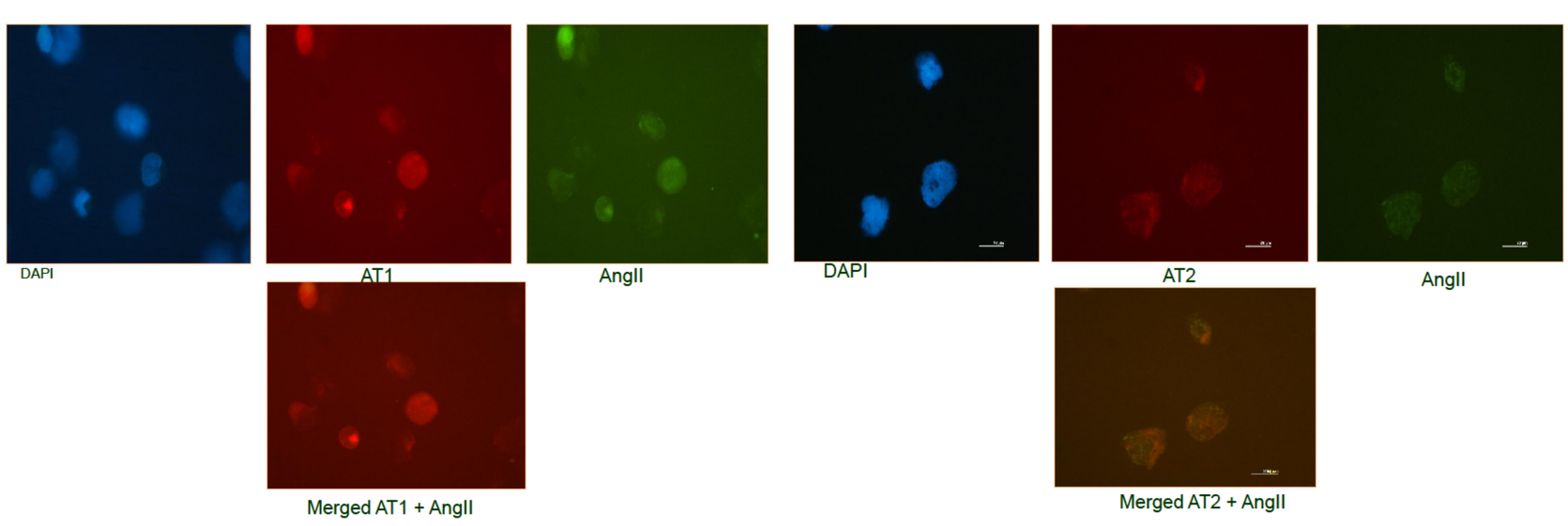
Immunofluorescences



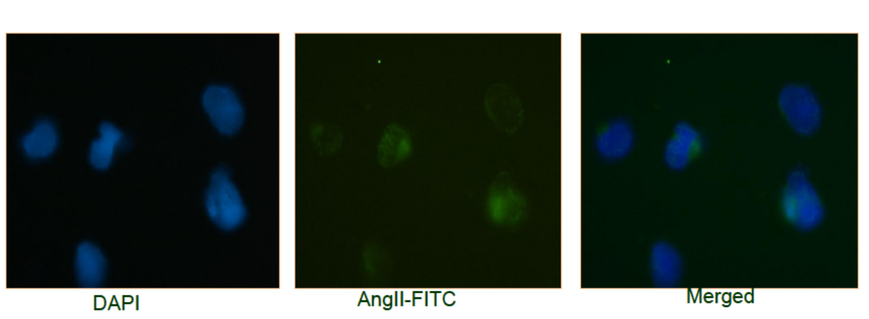
The distribution of AT1 and AT2 receptors in HMCs, staining with an anti-AT1antibody and a TRITC-labeled secondary antibody (red) and FITC-labeled secondary antibody (green). The nuclei are stained with DAPI.



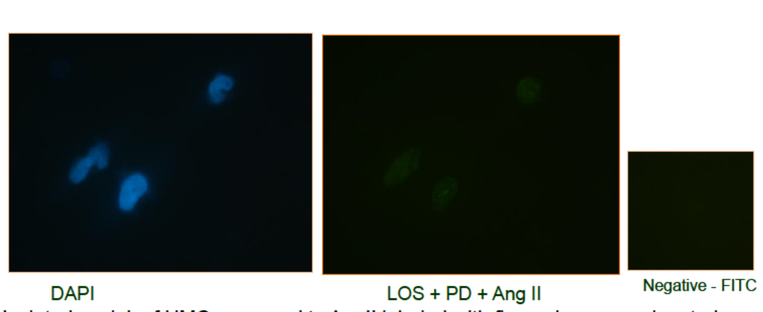
The distribution of AT1 and AT2 with Nup62 in isolated nuclei of HMCs, staining with an anti-AT1, anti-AT2 and Anti-Nup62 antibodies and a TRITC-labeled secondary antibody (red) and FITC-labeld secondary antibody (green). The nuclei are stained with DAPI, and the merged image is also shown.



The distribution of AT1 (A) and AT2 (B) with exogen Angiotensin II in isolated nuclei of HMCs, staining with an anti-AT1, anti-AT2 and Anti-Nup62 antibodies and a TRITC-labeled secondary antibody (red) and FITC-labeld secondary antibody (green). The nuclei are stained with DAPI, and the merged image is also shown.



Isolated nuclei of HMCs exposed to AngII labeled with fluorochrome-conjugated FITC (A) and losartan (AT1 blocker) and PD-123319 (AT2 blocker)ni. The nuclei are stained with DAPI.



Isolated nuclei of HMCs exposed to AngII labeled with fluorochrome-conjugated FITC (A) andlosartan (AT1 blocker) and PD-123319 (AT2 blocker). The nuclei are stained with DAPI.

Summary and Discussion

- ➢ The existence of receptors in the nuclear membrane of mesangial cells was shown by imunofluorescence by the binding of anti-AT1 and anti-AT2 antibodies. The location of these receptors was confirmed by co-localization of protein NUP62 nuclear membrane, in both AT1 and AT2 receptors.
- ➢ We observed the presence of AT1 and AT2 receptors in nuclear membrane of the mesangial cells.
- ➢ The addition of AngII and the effect of AT1 and AT2 antagonists indicate that these receptors are able to bind this peptide.
- ➢ This data suggest a direct intranuclear action of AngII in mesangial cells.

Conclusion

- ➢ HMC cells present AT1 and AT2 receptors in the nuclear membrane able to bind Ang II.
- ➢The actions of Ang II mediated by activation of these receptors are being investigated.

51ERA-EDTA
22--MP
B) Cell signalling. Cell growth control and related alterations (hypertrophy, hyperplasia and apoptosis) including neoplasia.
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