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Human Renal Mesangial Cell Activation Induced by Endothelin-1 or IgA Nephropathy Patient-Derived Immune Complexes is Blocked by Selective ETA Antagonist Atrasentan

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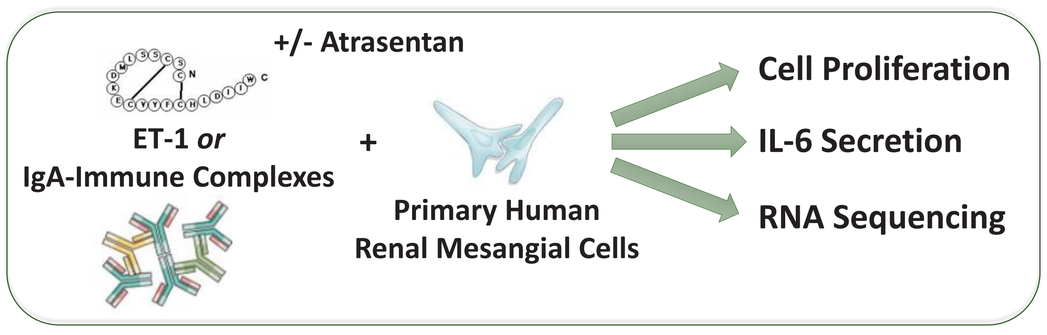
Background

- Mesangial cell activation in response to deposition of pathogenic IgA-containing immune complexes is characterized by cellular proliferation and inflammatory cytokine secretion and is considered the initiating intra-renal event in the pathogenesis of IgA nephropathy (IgAN)[1]. However, the molecular mechanisms have not been well defined.
- Endothelin (ET) pathway activation has been observed in kidney biopsies of IgAN patients and may be an important driver of disease progression by promoting proteinuria along with kidney inflammation and fibrosis via ETA receptor activation.
- Here, we investigate the role of the ETA receptor in mesangial cell activation in response to ET-1 and IgAN patient-derived immune complexes, using the selective ETA antagonist atrasentan[2].

Methods and Materials

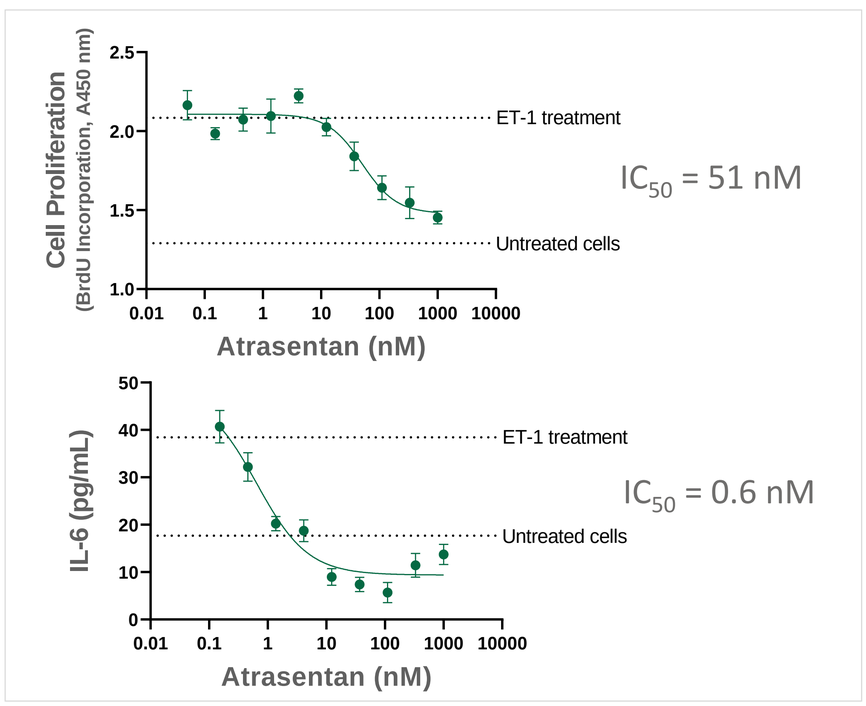
Primary human renal mesangial cells (HRMC) from ScienCell were cultured under standard conditions up to 4 passages. HRMCs were treated with ET-1 at 4 nM concentration for up to 72 hours in the presence or absence of atrasentan, after which proliferation and cytokine production were measured. Global transcriptional responses were characterized by RNA sequencing and qPCR following 24 hours.

IgA-containing immune complexes were purified from the serum of either IgAN patients or age and sex matched healthy controls using jacalin-agarose affinity chromatography[3]. HRMCs were cultured for 72 hours with IgA-containing immune complexes with or without atrasentan and HRMC proliferation was analyzed.



Results

A ET-1 induced increases in HRMC proliferation and IL-6 secretion are blocked by atrasentan in a concentration-dependent manner



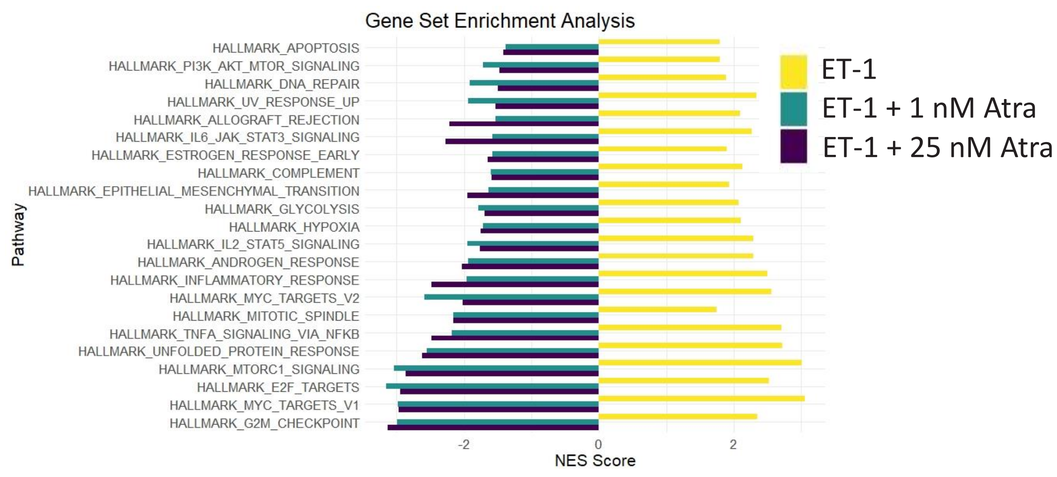
HRMCs were incubated for 72 hours with 4 nM ET-1; atrasentan blocked the increase in cell proliferation and IL-6 secretion with IC_{50} values of 51 nM and 0.6 nM, respectively

B Transcriptomic analysis reveals ET-1 regulation of multiple genes in HRMCs and reversal with atrasentan treatment

HRMCs were incubated with 4 nM ET-1 for 24 hours with or without 25 nM atrasentan and differential gene expression was determined following RNA sequencing

Results (continued)

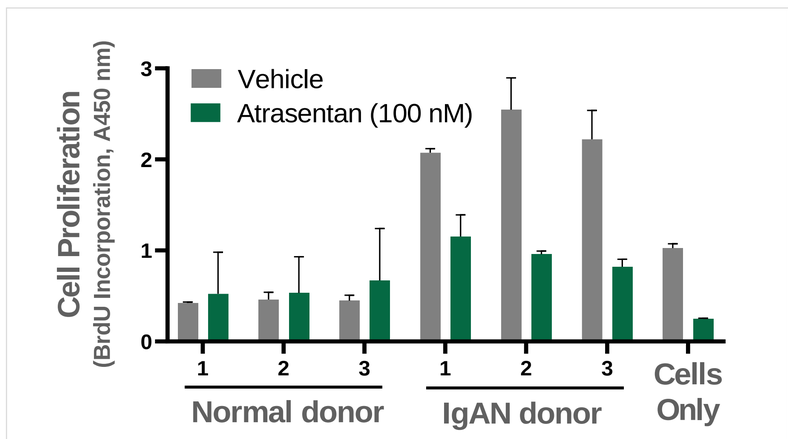
C Gene set enrichment analysis identifies upregulation of cell proliferation, pro-fibrotic and pro-inflammatory pathways with ET-1 treatment in HRMCs, which are blocked by atrasentan



D Serum from IgAN patients has elevated galactose deficient (Gd)-IgA compared to matched control serum

Patient ID	Age	M/F	Proteinuria (g/24 h)	GFR (ml/min/1.73m^2)	Total IgA (µg/mL)	Gd-IgA (µg/mL)
Normal 1	30-35	M	NA	NA	1107	2.7
Normal 2	30-35	F	NA	NA	296	1.4
Normal 3	30-35	F	NA	NA	354	1.7
				Mean ± SEM	**586 ± 261**	**1.9 ± 0.4**
IgAN 1	32	M	1.7	33	1229	3.3
IgAN 2	33	F	4.7	63	425	5.4
IgAN 3	37	F	2.2	48	1085	4.4
				Mean ± SEM	**913 ± 248**	**4.4 ± 0.6**

E Atrasentan prevents hyperproliferation of HRMCs in response to IgA-containing immune complexes purified from IgAN patients



IgAN patient immune complexes caused 5.1-fold increase in HRMC proliferation compared to normal donors following 72 hours treatment

Atrasentan significantly ($p<0.01$) attenuated proliferation induced by IgA-containing immune complexes purified from IgAN patients (57 ± 6% reduction)

Conclusion

- IgAN is characterized by mesangial cell proliferation associated with the deposition of pathogenic galactose deficient IgA-containing immune complexes and our studies support a role for ETA receptor activation contributing to this pathogenic mechanism.
- Exogenous ET-1 directly stimulates HRMC activation, including cell proliferation and upregulation of pro-inflammatory and pro-fibrotic pathways, which can be blocked by the ETA antagonist atrasentan.
- Atrasentan prevents HRMC hyperproliferation in response to IgA-containing immune complexes purified from IgAN patients, suggesting that the autocrine action of endogenously produced ET-1 on ETA receptors contributes to mesangial cell activation that results from pathogenic IgA-containing immune complexes.

These results support the therapeutic potential of atrasentan in IgAN patients, not only via its well characterized effect to reduce proteinuria, but also by potentially reducing mesangial cell activation, a hallmark of IgAN

- The Phase 3 ALIGN trial is assessing the efficacy, safety and tolerability of atrasentan in IgAN patients at risk of progressive kidney function loss, despite optimized RAS blockade. (ClinicalTrials.gov Identifier: NCT04573478)

ALIGN

WCN21-0848

References

1. Suzuki et al., *J. Am. Soc. Nephrol.* 22: 1795-1803, 2011
2. Wu-Wong et al., *Clin. Sci. (Lond.)* 103(48): 107s-111s, 2002
3. Novak et al., *Kidney Int*. 67(2): 504-13, 2005