KEYSTONE SYMPOSIA
Myeloid Cell Diversity: From Fundamental Biology to Disease States, 2024

Demonstration of an isoform-specific anti-inflammatory role for Neuropilin-2 through a novel interaction with the chemokine ligand 21

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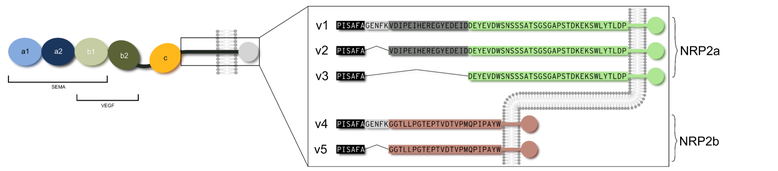
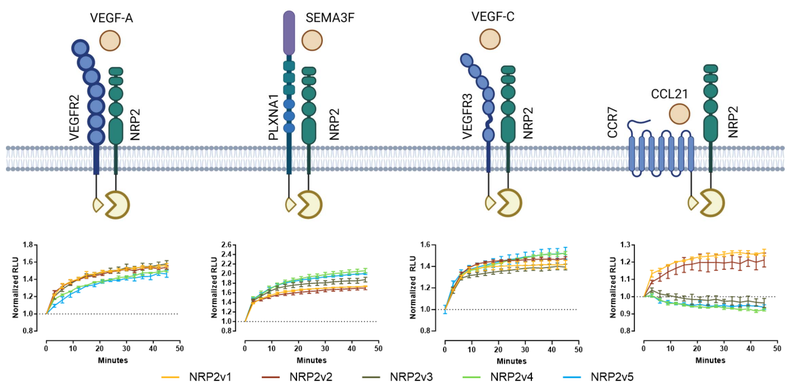
NRP2

Neuropilin-2 (NRP2), a cell surface receptor originally identified as a co-receptor for vascular endothelial growth factors and semaphorins, is translated into two protein isoforms with distinct C-terminal sequences termed NRP2a and NRP2b. Recent evidence suggests that both isoforms of NRP2 are expressed on various immune cell types, especially myeloid cells, and that expression is enhanced upon activation. Previous work has shown that mice with myeloid-specific ablation of NRP2 exhibited significant immune dysregulation[1,2], an effect we show in additional models of induced inflammation in a full NRP2 knockout mouse (both isoforms), including oxazolone induced atopic dermatitis and collagen-induced arthritis. This work indicates that NRP2 may play a role in regulating the myeloid immune response, however the functional significance of its different isoforms and their interactions with relevant co-receptors is not well understood.

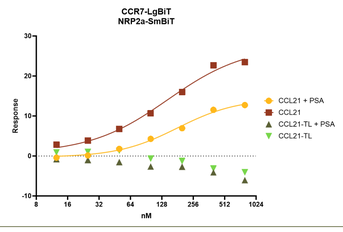
To better clarify the underlying molecular nature of the potential role of NRP2 in downregulating inflammation, we evaluated associations between NRP2 and its known and possible co-receptors through receptor association studies. We demonstrated and characterized a novel interaction between the 'a' isoform of NRP2 and the chemokine ligand CCL21, linking NRP2a, but not NRP2b, to the regulation of the central chemokine receptor CCR7. A truncated form of CCL21 has been described, where its C-terminal extended tail has been removed, and we show that NRP2a interacts specifically with the extended ligand but not its truncated form. Striking evolutionary co-conservation is seen between NRP2a and the extended tail of CCL21, corresponding with the emergence of lymph nodes. Blockade of this interaction with an NRP2a-specific antibody replicates the pro-inflammatory phenotypes seen in knockout animals.



CCL21 induces receptor association between CCR7 and NRP2a

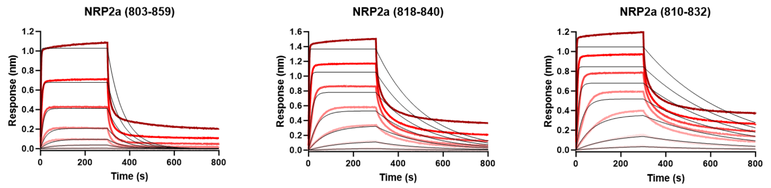


- Receptor association measured by NanoLuc complementation[3] of NRP2 isoforms with known co-receptors/ligands and CCR7/CCL21 showing isoform specificity of NRP2/CCR7 association.
- Association of NRP2 and CCR7 induced by full CCL21 ligand (aa24-134) but not a truncated tailless (TL) (aa24-102) form.
- Addition of enzyme responsible for polysialylation (PSA) changes magnitude of signal, but not dose responsiveness.



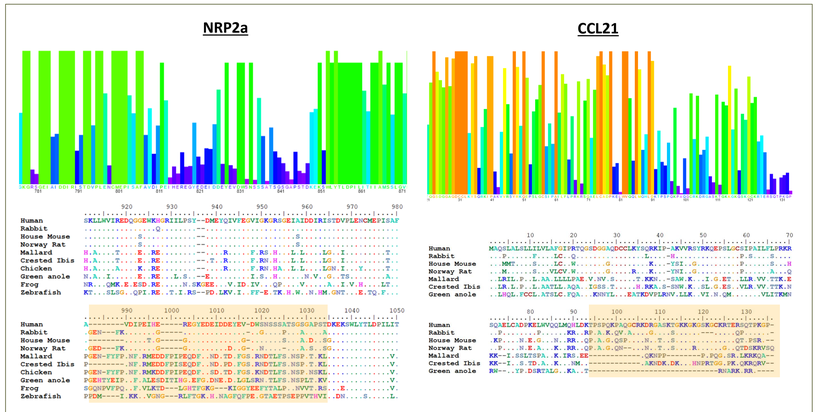
CCL21 binds to a minimal membrane proximal region of NRP2a

Peptide	Sequence	Affinity to CCL21
NRP2a(803-859)	PISAFAVDIPEIHER**EGYEDEIDDEYEVDW**SNSSSATSGSGAPSTDKEKSWLYTLDPK(Biotin)	30 nM
NRP2a(818-840)	--------------**EGYEDEIDDEYEVDW**SNSSSATS----------K(Biotin)	11 nM
NRP2a(810-832)	------DIPEIHER**EGYEDEIDDEYEVDW**----------K(Biotin)	4 nM

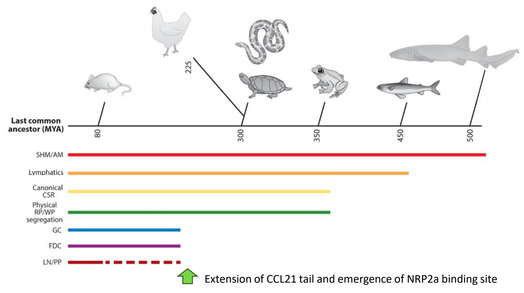


- Binding of CCL21 to a series of peptides representing the membrane proximal region of NRP2a.

Unique interacting domains of NRP2a and CCL21 evolved in parallel



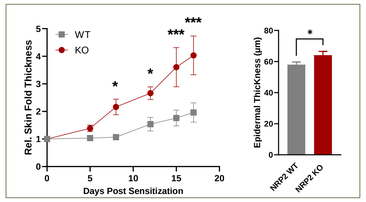
- Sequence conservation[4] of all mammalian orthologues of NRP2 and CCL21 show distinct regions of conservation in identified binding site in NRP2a, and similarly a region of conservation in the CCL21 tail.
- Alignment to non-mammalian orthologs shows alternative splicing disturbing both the binding site in NRP2 and loss of the CCL21 tail in non lymph node containing animals.



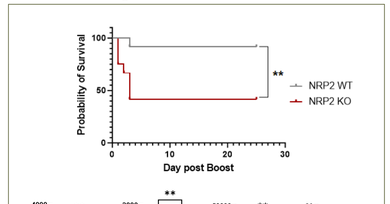
NRP2a blockade replicates pro-inflammatory phenotype of NRP2 knockout

Total NRP2 knockout

Oxazolone induced atopic dermatitis



Collagen induced arthritis



- NRP2 KO and WT mice were sensitized with oxazolone in a model of atopic dermatitis, in-life and terminal histology of skin thickness showed enhanced inflammation.
- NRP2 KO and WT mice were immunized with collagen as a model of arthritis resulting in poor survival of KO mice post boost. 48 hours post boost elevation of pro-inflammatory cytokines was seen.

NRP2a antibody blockade

Oxazolone induced atopic dermatitis

Collagen induced arthritis

An antibody blocking CCL21 binding to NRP2a was used in the same models of disease resulting in:
- An increase in skin thickness by IHC and spleen weights relative to IgG control in induced atopic dermatitis.
- Acceleration of disease development and reduction in circulating levels of TGF-β1 in collagen induced arthritis.

Conclusions

- NRP2a and CCR7 receptor proximity increases in the presence of CCL21.
- The elements required for interaction between NRP2a and CCL21 show evolutionary conservation.
- NRP2a blockade replicates pro-inflammatory phenotype of NRP2 knockout in models of inflammation.
- These results suggest NRP2 may play a key immune regulatory role through its association with CCR7.

References:
1. Immormino RM, et al. Neuropilin-2 regulates airway inflammatory responses to inhaled lipopolysaccharide. Am J Physiol Lung Cell Mol Physiol. 2018
2. Li T, et al. Deficiency of inflammation-sensing protein neuropilin-2 in myeloid-derived macrophages exacerbates colitis via NF-κB activation. J Pathol. 2023
3. Dixon AS, et al. NanoLuc Complementation Reporter Optimized for Accurate Measurement of Protein Interactions in Cells. ACS Chem Biol. 2016
4. Lua RC, et al. UET: a database of evolutionarily-predicted functional determinants of protein sequences that cluster as functional sites in protein structures. Nucleic Acids Res. 2016
5. Neely HR, Flajnik MF. Emergence and Evolution of Secondary Lymphoid Organs. Annu Rev Cell Dev Biol. 2016

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