1785

Domain-specific Antibodies to Neuropilin-2 Implicate VEGF-C and Not Semaphorin 3F in Breast Cancer Stem Cell Function

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Abstract

INTRODUCTION: There is a strong body of evidence indicating that the expression of Neuropilin-2 (NRP2) is enriched in breast cancer stem cells (CSCs) and that NRP2 signaling is critical for breast CSC function and resistance development. For this reason, the rationale for targeting NRP2 as a therapeutic strategy is compelling and timely. A major limitation that has hampered the development of such a therapy, however, has been the lack of availability of high-quality anti-human NRP2 monoclonal antibodies (mAbs) that block NRP2 signaling.

aTyr Pharma has generated a panel of high-quality, anti-human NRP2 mAbs that have the potential to be developed for the clinical management of a variety of diseases including cancer and inflammation. A significant advance made by aTyr is that through specific domain reactivity, they have demonstrated differential effects on ligand blocking, receptor homo and heterodimerization and functional activity. Importantly a subset of such antibodies show differential activity in the mammosphere assay of triple negative breast cancer.

RESULTS: Flow cytometry was used to assess the specificity of the aTyr anti-NRP2 mAbs to NRP2 using A549 wild type versus NRP2 knockout clonal cells. The aTyr anti-NRP2 mAbs bound to A549 wild-type cells while showing little or no binding to the NRP2 knockout clonal cells, exhibiting significantly superior specificity and sensitivity compared to existing commercially available antibodies. Displacement studies demonstrated that the tested anti-NRP2 mAbs showed different capabilities in blocking of VEGF-C or SEMA-3F binding to Expi293-hNRP2 cells, and were categorized as blockers (>90% inhibition), partial blockers (30-90% inhibition), or non-blockers (no obvious inhibition).

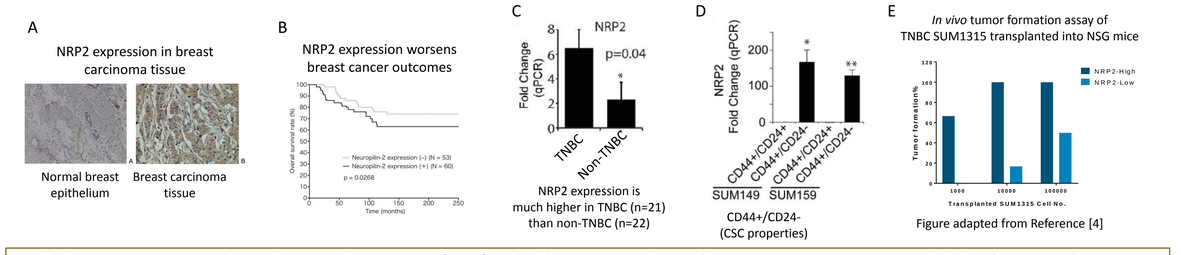
To further extend the assessment of the biological activity of the anti-NRP2 antibodies, their activity was assessed in a receptor dimerization assay. Vectors encoding a split luciferase, and a cell permeable substrate, were obtained from Promega corporation. The complete extracellular domain and transmembrane helices of NRP2, FLT4 (VEGFR3), KDR (VEGFR2) and plexin A1 (PLXNA1) were cloned into the vectors and screened for optimal orientation, following established methods. Tested antibodies were able to impair respective VEGF and SEMA3 induced dimerization of receptor pairs NRP2/FLT4, NRP2/KDR and NRP2/PLXNA1. Select antibodies show extremely specific and non-obvious functional differentiation.

Direct functional assessment of a subset of these antibodies on breast CSCs revealed that the VEGF blocking, but not the SEMA3 blocking anti-NRP2 mAbs had the ability to inhibit serial passage mammosphere formation, an indicator of self-renewal potential.

CONCLUSIONS: aTyr has developed and characterized a series of domain specific antibodies to NRP2. These antibodies show differential binding to specific domains of NRP2, can inhibit either VEGF-C or Sema3F binding to NRP2, and differentially effect receptor dimerization. The use of these antibodies enabled us to implicate VEGF-C/NRP2 signaling but not SEMA-3F/NRP2 signaling in the function of breast CSCs.

Introduction

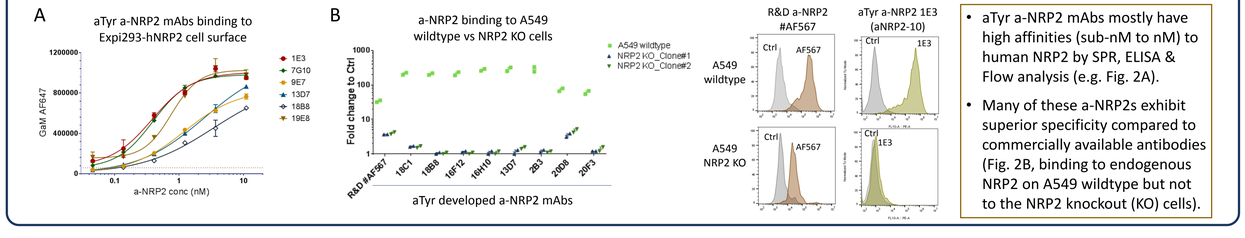
Figure 1. NRP2 Association with Breast Cancer and Enrichment in Breast CSCs



- Published data demonstrate that triple negative breast cancer (TNBC) is a highly aggressive subtype of breast cancer, and contains a much higher frequency of cancer stem cells (CSCs) than other subtypes, which may be responsible for poor patient outcomes by promoting therapy resistance, metastasis, and recurrence [1, 2].
- NRP2 is enriched in TNBC and breast CSCs (Fig. 1). VEGF-NRP2 signaling promotes stem-like traits in breast cancer cells (e.g. tumor formation *in vivo,* Fig. 1E, [3-5])

Results

Figure 2. aTyr Developed a-NRP2 mAbs Exhibit Superior Specificity and Sensitivity Compared to Commercially Available Antibodies



- aTyr a-NRP2 mAbs mostly have high affinities (sub-nM to nM) to human NRP2 by SPR, ELISA & Flow analysis (e.g. Fig. 2A).
- Many of these a-NRP2s exhibit superior specificity compared to commercially available antibodies (Fig. 2B, binding to endogenous NRP2 on A549 wildtype but not to the NRP2 knockout (KO) cells).

Reference

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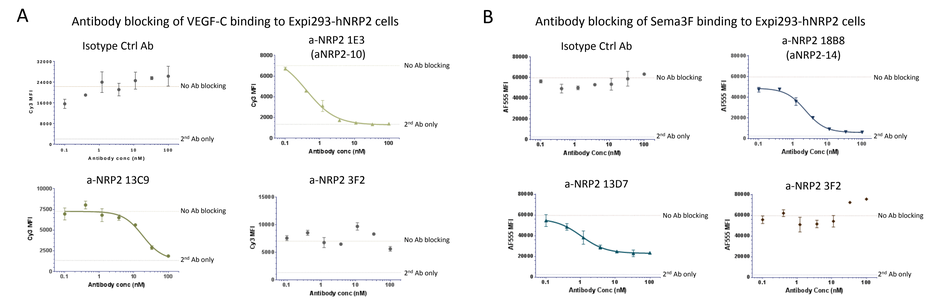
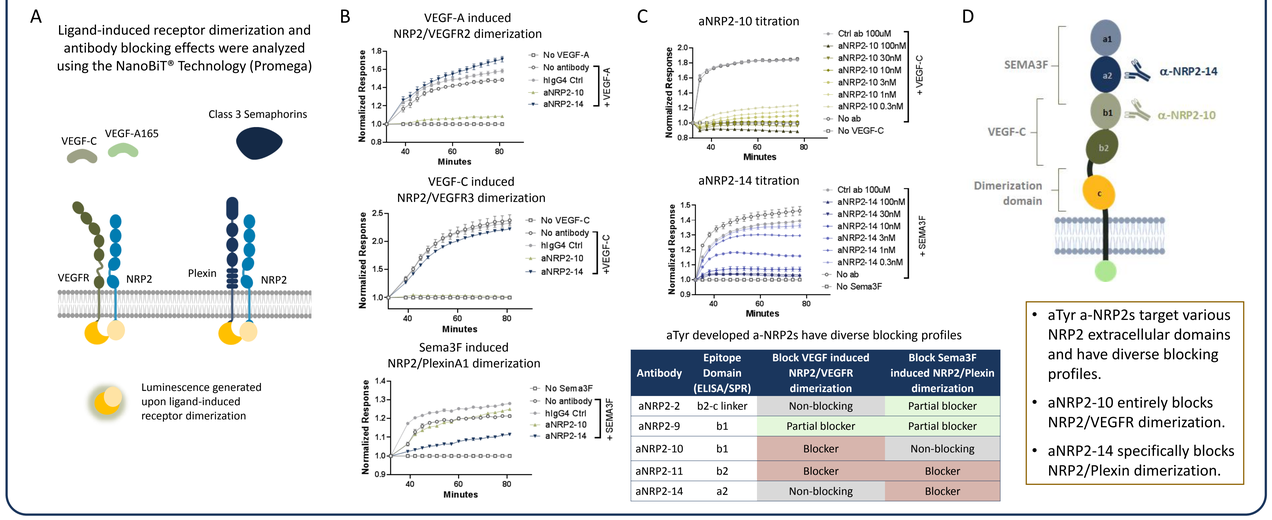
Figure 3. aTyr a-NRP2 Blocking of Ligand Binding to Expi293-hNRP2 Cells



- aTyr a-NRP2s showed different capabilities in blocking of VEGF-C or SEMA-3F binding to Expi293-hNRP2 cells, including complete, partial or non-blockers (Fig. 3).
- aNRP2-10 blocks VEGF-C binding to Expi293-hNRP2 cells (Fig. 3A).
- aNRP2-14 blocks Sema3F binding to Expi293-hNRP2 cells (Fig. 3B).

Figure 4. aTyr a-NRP2 Blocking of Ligand Induced NRP2/co-receptor Dimerization

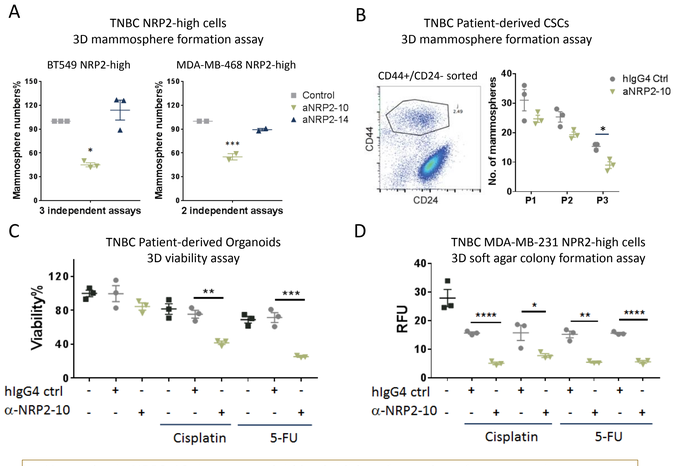


aTyr developed a-NRP2s have diverse blocking profiles

Antibody	Epitope Domain (ELISA/SPR)	Block VEGF induced NRP2/VEGFR dimerization	Block Sema3F induced NRP2/Plexin dimerization
aNRP2-2	b2-c linker	Non-blocking	Partial blocker
aNRP2-9	b1	Partial blocker	Partial blocker
aNRP2-10	b1	Blocker	Non-blocking
aNRP2-11	b2	Blocker	Blocker
aNRP2-14	a2	Non-blocking	Blocker

- aTyr a-NRP2s target various NRP2 extracellular domains and have diverse blocking profiles.
- aNRP2-10 entirely blocks NRP2/VEGFR dimerization.
- aNRP2-14 specifically blocks NRP2/Plexin dimerization.

Figure 5. aNRP2-10 Showed Tumor-inhibitory Effects on Cells or Organoids from TNBC Patients



- Humanized aNRP2-10 decreased viability, inhibited formation of mammospheres and anchorage-independent growth in cells or organoids from TNBC patients.
- Effects of aNRP2-10 are more pronounced in combination with chemotherapy agents.

(*p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001 significantly different from control)

Conclusions

- **aTyr has generated a panel of high-quality, anti-human NRP2 mAbs that have the potential for clinical development.**
- **aTyr a-NRP2 mAbs exhibit superior specificity and sensitivity compared to commercially available antibodies.**
- **aTyr a-NRP2 mAbs have diverse profiles in blocking of ligand binding and ligand-induced NRP2/co-receptor dimerization.**
- **aTyr aNRP2-10 specifically blocks VEGF-binding to NRP2 and VEGF-induced NRP2/VEGFR dimerization.**
- **aTyr aNRP2-14 specifically blocks Sema3F-binding to NRP2 and Sema3F-induced NRP2/Plexin dimerization.**
- **aTyr aNRP2-10 showed tumor-inhibitory effects on TNBC CSCs or organoids, demonstrating the potential to be developed for the clinical management of breast cancer.**

Acknowledgements

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