RGMc-selective antibodies modulate iron homeostasis in vivo

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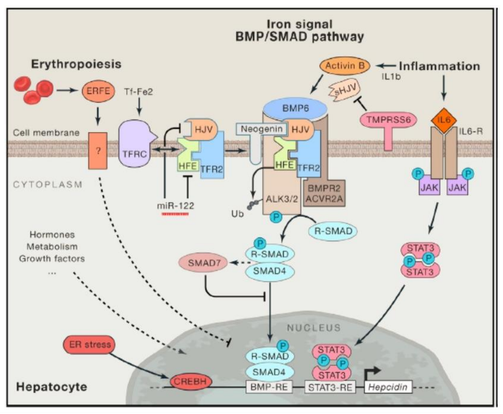
Abstract

Currently approved treatments for anemia, including intravenous iron and erythropoiesis stimulating agents (ESA's), are suboptimal, and have been associated with significant safety risks. Hepcidin, a peptide produced by the liver, acts as a negative regulator of systemic iron availability by inducing the degradation of the iron exporter protein, ferroportin. Hepcidin levels are elevated by inflammation, which leads to functional iron deficiency and iron-restricted anemia in many disease settings. Repulsive Guidance Molecule c (RGMc) is a liver-expressed BMP co-receptor that is required for BMP-6 induction of hepcidin expression in the liver. By targeting RGMc and blocking BMP-6 signaling it is possible to modulate hepcidin levels and potentially overcome disease associated iron restriction, while minimizing possible toxicities associated with systemic inhibition of the RGMa/b axis. In this study, highly RGMc-selective antibodies were discovered , affinity matured, and shown to block BMP-6 interaction with RGMc, selectively repressing the BMP6-hepcidin axis and durably increasing the availability of iron. The resulting high affinity RGMc antibodies had no detectable cross-reactivity with other protein family members, RGMa or RGMb. A model for target selectivity of these antibodies was generated using Hydrogen-Duterium exchange (HDX) mapping and revealed that one subset of the antibodies had an epitope in the BMP binding domain of RGMc. By selectively targeting BMP-6 signaling in the liver there is an opportunity to treat iron restricted anemias in a variety of chronic inflammatory diseases.

Figure 1. RGMc is an important co-receptor in the hepcidin signaling pathway



RGMc also know as Hemojuvulin (HJV) is a BMP-6 co-receptor critical for signaling in the hepcidin iron metabolism pathway.

Anemia of Inflammation/ Chronic Disease	
Untreated	Ab treated
↑ Hepcidin	↓ Hepcidin
↓ Serum iron	↑ Serum iron

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Figure 2. RGMc Adimab yeast-based platform campaign workflow

Adimab yeast-based platform screened for human and mouse RGMc binding and deselected on human RGMa and -b

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Panel of RGMc-selective antibodies sampling broad epitope space identified

↓

Low affinity clones were screened for function

↓

BMP-6 competitive antibodies identified in vitro

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Adimab yeast-based platform affinity maturation of 4 BMP6 competitive clones

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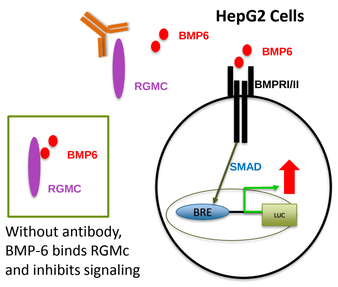
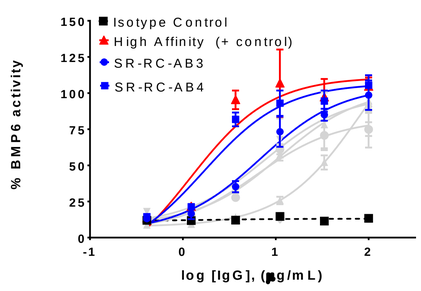
High affinity clones rescreened in vitro & in vivo

Figure 3a. BMP-6 competitive antibodies from naïve campaign identified in vitro

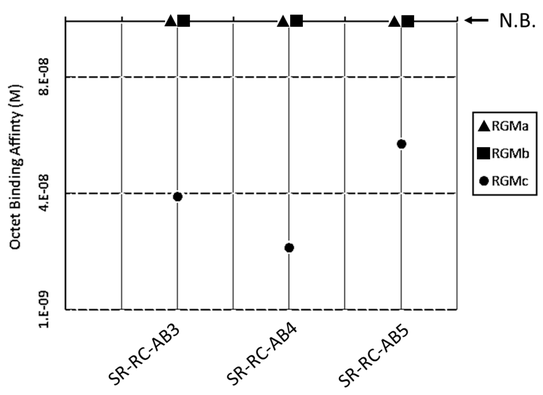




- BMP-6 Competition was measured by a BRE-Luciferase activity
- 11 antibodies identified to be competitors of BMP-6 binding on RGMc

Exogenous BMP-6 and RGMc are added to HepG2 cells with the BMP Responsive Element (BRE) luciferase reporter. BMP-6 competitive antibodies compete for RGMc binding allowing the BMP-6 to signal through the luciferase reporter. Measurement of antibody activity is represented as percentage of BMP-6 signaling.

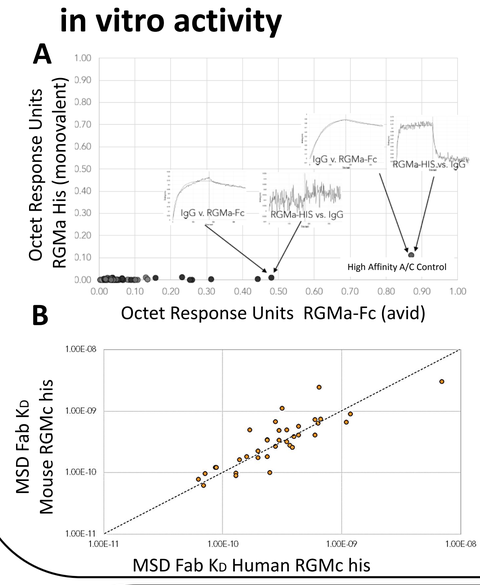
Figure 3b. Naïve antibodies show specificity for RGMc over RGMa and -b



- No significant binding to RGMa or –b was measured
- Affinities ranged from double digit to triple digit nanomolar for the RGMc-selective clones

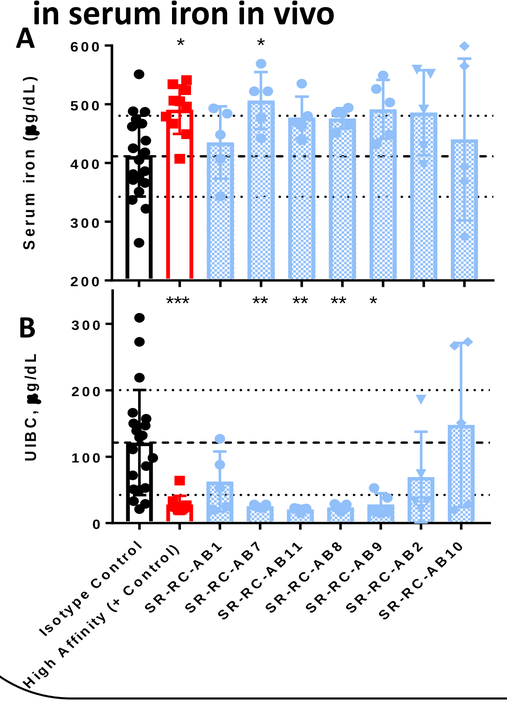
- Four BMP-6 competitive clones were selected for affinity maturation using Adimab yeast-based platform
- Deselection Rounds using RGMa were continued through affinity maturation to avoid gaining cross-reactivity

Figure 4. Affinity Maturation yields RGMc-selective antibodies with improved in vitro activity



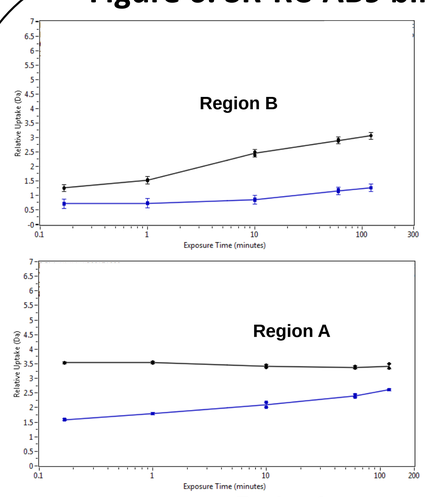
- RGMc specificity was maintained for most families in the affinity matured progeny (A)
- One family of clones had some progeny with measurable binding to an avid form of RGMa (A)
- Affinities were similar for human and mouse RGMc (B)
- All affinity matured antibodies showed improvement from parental sequence in vitro (C & D)

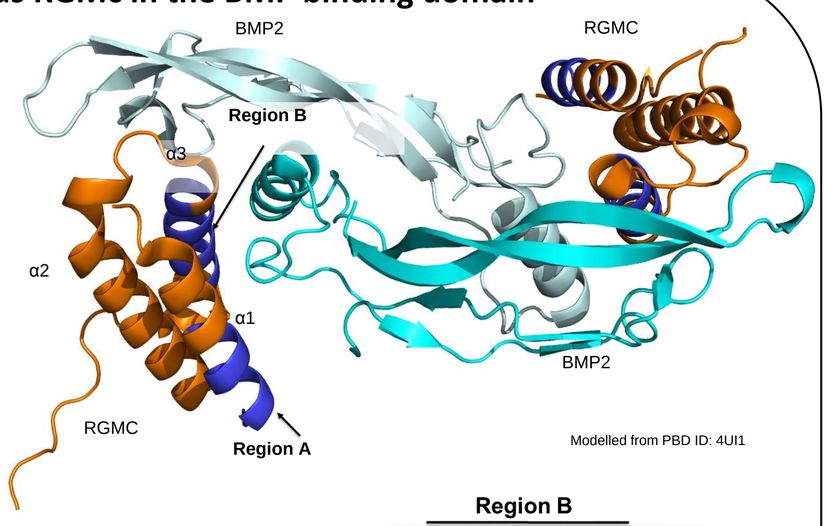
Figure 5. Affinity Matured RGMc-selective antibodies show potent increase in serum iron in vivo



- BMP6 competitive affinity matured antibodies showed an increase in serum iron and a decrease in the UIBC after 24hs (0.2 mpk single dose) (A & B)
- High affinity antibodies showed up to 3 weeks prolonged increase in serum iron in SD rats after a single 20 mpk dose (C)

Figure 6. SR-RC-AB9 binds RGMc in the BMP binding domain





Region A

RGMC AEYVSSTLSLRGGGSSGALRGGGGGGRGGGV
RGMA SEFWSATSGSHAPA----------SD
RGMB TDFVSLTSHLNSAV----------DG

Region B

RGMC LAFHSAVHGIEDLMIQH
RGMA LAYHSAVHGIEDLMSQH
RGMB LVYHSAVLGISDLMSQR

- HDX experiments showed that when bound to the Fab fragment of SR-RC-AB9 two peptides showed significant protection
- These peptides map to two of the three helices in the BMP6 binding domain of RGMc, indicating the epitope
- Of these two regions the first (region A) is a region of significant difference across the RGM family which could contribute to the RGMc specificity of the antibody

Table 1. Affinity matured antibodies reached sub-nanomolar K_D values

		K_D (M)	EC50 (ug/mL)
Parental	SR-RC-AB3	4.30E-9	4.83
	SR-RC-AB4	4.00E-9	5.25
Affinity Matured	SR-RC-AB7	2.10E-11	3.87
	SR-RC-AB8	3.90E-11	3.63
	SR-RC-AB9	2.40E-11	3.45

- Affinity measurements determined by MSD-SET
- Significant improvement in affinities to RGMc

Conclusion

We demonstrate that targeted inhibition of the BMP6-hepcidin pathway by RGMc-selective antibodies increases the availability of iron in vivo. SR-AB9 directly competes with BMP-6 by binding with high affinity in the N-terminal BMP binding domain of RGMc. This inhibition results in a decrease in serum hepcidin levels and a sustained increase in serum iron levels in rats. We believe that liver-selective inhibition of BMP6 signaling could provide a safe and effective way to target a variety of iron-restricted anemias.

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Conflict of Interest

[1] Current Employee and Shareholder of Scholar Rock.
[2] Former employee and Shareholder of Scholar Rock