Mechanism of galectin-3 binding by belapectin, a galectin-3 inhibitor developed for NASH cirrhosis

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Belapectin, a galectin-3 inhibitor, is currently in phase 2b/3 development for the prevention of esophageal varices in patients with NASH cirrhosis[1].

Belapectin is a large polycarbohydrate molecule that is primarily captured by activated macrophages and thus inhibits galectin-3 at its main site of production.

Galectin-3, a glycan binding protein, plays a central role to foster liver inflammation and fibrosis in NASH cirrhosis and create the so-called 'galectin-3 *fibrosome*.'

We evaluated the intimate binding of belapectin to galectin-3 using heteronuclear Nuclear Magnetic Resonance (NMR) spectroscopy.

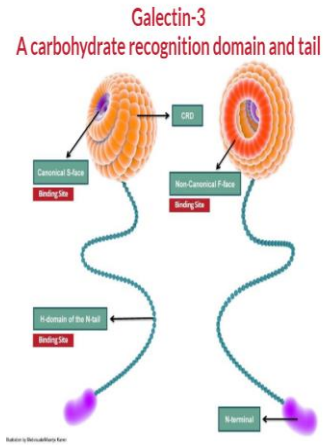
Galectin-3 was expressed in BL21 (DE3) cells (Novagen), a cell competent for high protein expression, grown in minimal media, purified over a lactose affinity column, and fractionated on a gel filtration column, as described previously for galectin-1[2].

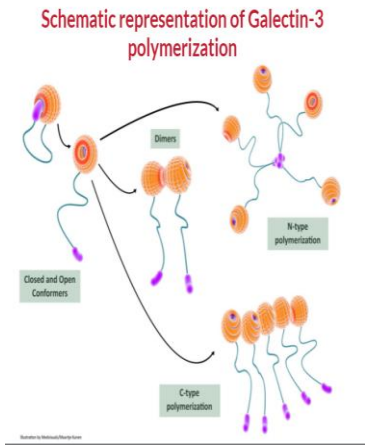
Purity was established by SDS PAGE and mass spectrometry.

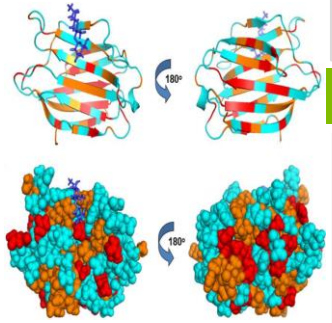
NMR experiments for the binding of belapectin to recombinant human galectin-3 was conducted as previously reported[3].

Raw data were converted and processed by using NMRPipe data processing software and were visualized and analyzed with NMRview.

- In presence of belapectin, there were significant perturbations at both the carbohydrate recognition domain and N-terminal domain of galectin-3 (figure: left and center panel).
- Within the carbohydrate recognition domain, interactions were multi-modal. Resonances shifted at residues in β-strands 3 and 4 on the S-face of the canonical glycan-binding face and in β-strands 2, 8, 9 and 11 of the F-face (figure: right panel).
- Within the NT, interactions were concentrated at the N-terminus. Sequences 2-9 and 21-35 were most affected and included L7, H8, G15, A31, GAG34, A39, S40, Q48. Although residues within the C-terminal part of the NT were least affected, a few residues around G65 and S84 were perturbed.
- As the titration with belapectin continued, all resonances eventually become significantly broadened.
- The binding stoichiometry indicated about 5 molecules of galectin-3 per one molecule of belapectin.







Galectin-3 CRD/belapectin interaction

Red: large amino acid shifts, **Orange**: moderate shift, **Navy Blue**: scaffold of natural ligand.

By its molecular composition and its large molecular weight, belapectin is able to bind and interact with galectin-3 at multiple levels.

On the C-terminal part, belapectin binds to the S-face and F-face of the galectin-3 carbohydrate recognition domain that are the natural ligand-binding domains.

On the N-terminal part, belapectin also perturbs the tail structure, a structure that plays a role in its polymerization and the build up of the galectin-3 *fibrosome*.

With these multiple interactions, belapectin not only interferes with the binding function of galectin-3 but may also impact its 3-dimensional spatial configuration and plasticity.

These plethoric interactions, as well as the stoichiometry, may have translational advantages and may differentiate belapectin's mode of action from small molecule inhibitors of galectin-3 that exclusively target the S-face carbohydrate binding domain.

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3. Ippel H, Miller MC, Berbis MA, et al. (1)H, (13)C, and (15)N backbone and side-chain chemical shift assignments for the 36 proline-containing, full length 29 kDa human chimera-type galectin-3. Biomol NMR Assign 2015 Apr;9(1):59-63.

AFFILIATION/ACKNOWLEDGEMENT

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