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Dissertation Defense

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Fbg α C 389 – 402 MODULATES FACTOR XIII CROSSLINKING IN THE FIBRINOGEN α C REGION

ABSTRACT:

Fibrinogen (Fbg) is a coagulation protein critical for clot formation. Coagulation Factor XIII (FXIII) is a calcium-dependent transglutaminase that crosslinks reactive glutamines (Q) and lysines (K) between fibrin and other anti-fibrinolytic proteins.¹ In the presence of Ca^{2+} , FXIII can be activated non-proteolytically (FXIII-A^o), or proteolytically by thrombin (FXIII-A*²). Significant increases in clot stability and red blood cell retention are linked to FXIII activity in the fibrinogen α C region (Fbg A α 221 – 610).³ This region contains several FXIII-reactive glutamines and lysines, as well as a binding site for FXIII-A* (Fbg α C 389 – 402) that includes a key binding residue, Fbg α C E396.^{4,5} While FXIII-crosslinked clots maintain hemostasis, they also exacerbate the development of deep vein thrombosis (DVT).⁶ The work from this research seeks to aid further drug design against DVT by inhibition of FXIII binding and activity on Fbg α C.

Fbg α C 233 – 425, a “model” α C system that contains three reactive glutamines and the FXIII binding site (Fbg α C 389 – 402), was recombinantly expressed and purified. A series of mutations were subsequently introduced to the α C FXIII binding site to observe how crosslinking was affected. FXIII activity was monitored through mass spectrometry-based glycine ethyl ester (GEE) crosslinking and SDS-PAGE monodansylcadaverine (MDC) fluorescence crosslinking assays.⁷⁻⁸ Fbg α C 389 – 402 was found to selectively enhance Fbg α C crosslinking from FXIII-A* over FXIII-A^o.⁷ Additionally, Fbg α C 389 – 402 was more instrumental than either α C 403 – 425 or α C 328 – 388 in facilitating FXIII-A* crosslinking.⁸ Further work explored α C E395, D390, W391, and F394A as residues within Fbg α C 389 – 402 that could enhance α C FXIII-A* activity by increasing binding affinity. While E395 minimally impacted FXIII-A* activity, α C D390, W391 and F394 were subsequently identified as key residues alongside E396 for promoting FXIII-A* crosslinking.⁸ In summary, Fbg α C 389 – 402 was demonstrated to be a major facilitator of FXIII-A* activity on Fbg α C, as well as a potential target for therapeutic inhibition of VTE. Groundwork was laid for future studies through expression and preliminary crosslinking studies on Fbg α C 221 – 425, a new recombinant Fbg α C.

REFERENCES:

1. Schroeder, V.; Kohler, H. P., Factor XIII: Structure and Function. *Semin Thromb Hemost* 2016, 42 (4), 422-8.
2. Muszbek, L.; Bereczky, Z.; Bagoly, Z.; Komaromi, I.; Katona, E., Factor XIII: a coagulation factor with multiple plasmatic and cellular functions. *Physiol Rev* 2011, 91 (3), 931-72.
3. Byrnes, J. R.; Duval, C.; Wang, Y.; Hansen, C. E.; Ahn, B.; Mooberry, M. J.; Clark, M. A.; Johnsen, J. M.; Lord, S. T.; Lam, W. A.; Meijers, J. C.; Ni, H.; Ariens, R. A.; Wolberg, A. S., Factor XIIIa-dependent retention of red blood cells in clots is mediated by fibrin alpha-chain crosslinking. *Blood* 2015, 126 (16), 1940-8.
4. Schmitt, L. R.; Henderson, R.; Barrett, A.; Darula, Z.; Issaian, A.; D'Alessandro, A.; Clendenen, N.; Hansen, K. C., Mass spectrometry-based molecular mapping of native FXIIIa cross-links in insoluble fibrin clots. *J Biol Chem* 2019, 294 (22), 8773-8778.
5. Smith, K. A.; Adamson, P. J.; Pease, R. J.; Brown, J. M.; Balmforth, A. J.; Cordell, P. A.; Ariens, R. A.; Philippou, H.; Grant, P. J., Interactions between factor XIII and the alphaC region of fibrinogen. *Blood* 2011, 117 (12), 3460-8.
6. Wolberg, A. S.; Sang, Y., Fibrinogen and Factor XIII in Venous Thrombosis and Thrombus Stability. *Arterioscler Thromb Vasc Biol* 2022, 42 (8), 931-941.
7. Mohammed, R. D. S.; Ablan, F. D. O.; McCann, N. M.; Hindi, M. M.; Maurer, M. C., Transglutaminase Activities of Blood Coagulant Factor XIII Are Dependent on the Activation Pathways and on the Substrates. *Thromb Haemost* 2023, 123 (4), 380-392.
8. Ablan, F. D. O.; Maurer, M. C., Fbg alphaC 389-402 Enhances Factor XIII Cross-Linking in the Fibrinogen alphaC Region Via Electrostatic and Hydrophobic Interactions. *Biochemistry* 2023, 62 (14), 2170-2181.