Deciphering Discoidin Domain Receptor 1 Point Mutations Resulting in Constitutive Activation

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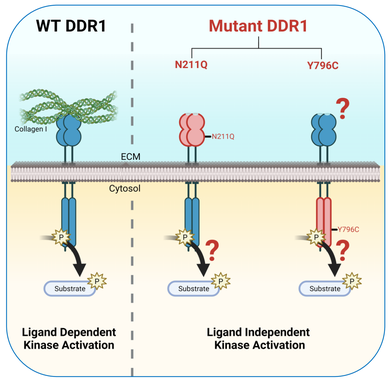


Highlights

Preliminary results:

- Impairing amino acids located in the DS-like domain (Asn211) and the kinase domain (Tyr796) of DDR1 differentially regulate DDR1 activation
- Asn211Gln mutation results in ligand independent DDR1 activation
- Tyr796Cys results in ligand independent downstream signaling activation.

Introduction & Background

Discoidin Domain Receptor 1 (DDR1) is a receptor tyrosine kinase (RTK) that is activated by collagens in the extracellular matrix[1,2]. Upon collagen activation, DDR1 undergoes tyrosine autophosphorylation in its kinase domain (KD), initiating various downstream signaling pathways vital for physiological and pathological functions such as cell migration, extracellular matrix remodeling and production, and inflammatory cytokine secretion[1,2,3]. Hence, the regulation of DDR1 activation is critical to prevent DDR1-mediated pathological effects.

The *N*-glycosylation site Asn211 in the Discoidin-like (DS-like) domain of DDR1 plays a role in maintaining the inactive state of the KD[4]. Additionally, Tyr796 in the KD has been suggested to play an autoinhibitory role on DDR1 activation. Though, whether the mutations Asn211Gln and Tyr796Cys result in ligand independent activation of DDR1 and contribute to its downstream signaling is yet to be determined. Here, we show that DDR1 carrying the Asn211Gln mutation undergoes autophosphorylation independent of collagen stimulation but does not activate its downstream signaling. In contrast, DDR1 carrying the Tyr796Cys mutation seems to initiate ligand independent downstream signaling but not receptor activation. Hence, preliminary results indicate that impairing the *N*-glycosylation site of the DS-like domain results in the constitutive activation of DDR1 and that Tyr796Cys DDR1 seemingly promotes downstream signaling.

Discoidin Domain Receptor 1 Signaling

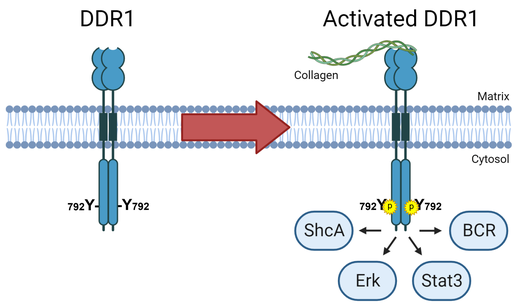


Figure 1. *Discoidin Domain Receptor 1 Signaling*. DDR1 is activated by collagens in the extracellular matrix. After collagen stimulation, DDR1 undergoes tyrosine autophosphorylation, initiating various downstream signaling pathways responsible for physiological function.

DDR1 Structure and Autoregulation

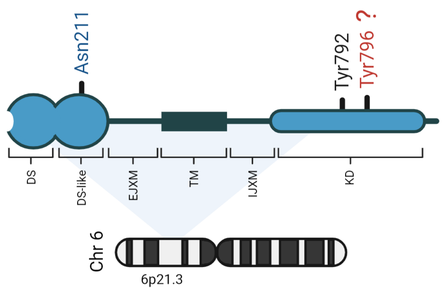


Figure 2. *Key Amino Acids that Regulate DDR1 Activation*. The glycosylation site Asn211 of the DS-like domain of DDR1 plays a role in maintaining the inactive state of the tyrosine kinase. Likewise, Tyr796 of the KD may exhibit an autoregulatory role in the kinase activation. Additionally, phosphorylation of Tyr792 of the KD indicates DDR1 activation.

Hypothesis

Do the amino acids Asn211 and Tyr796 negatively regulate tyrosine kinase activation of DDR1 and its downstream signaling?

Experimental Approach

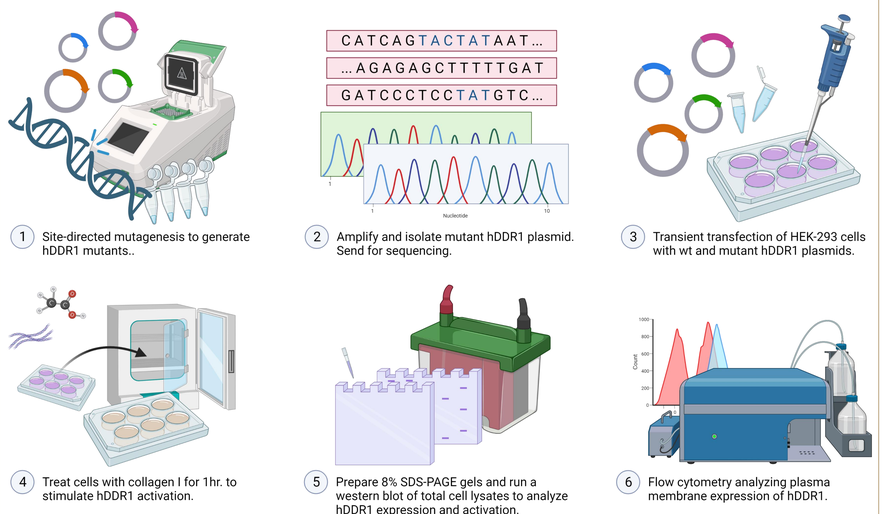


Figure 3. *Experimental Design Determining Mutant DDR1 Activation*. Mutant DDR1 plasmids were generated, sequenced, and transfected into HEK-293 cells. The cells were treated with collagen and analyzed by flow cytometry and lysed for western blot analysis.

pIRES Vector and Restriction Enzyme Digestion

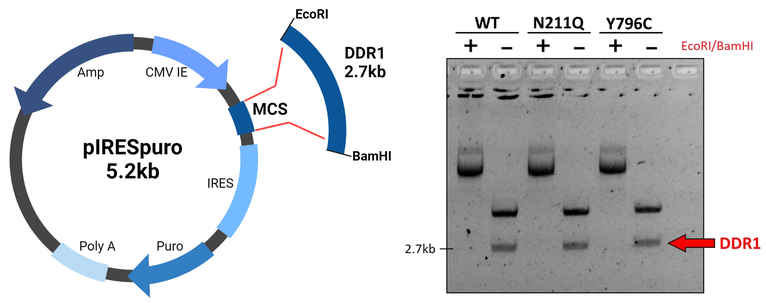
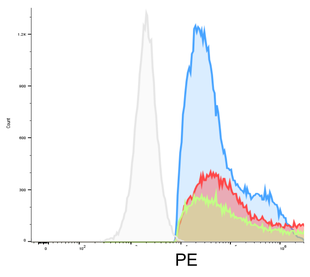


Figure 4. *Restriction Endonuclease Digest of pIRES hDDR1 Constructs*. pIRESpuro plasmid containing wild-type DDR1, N211Q, and Y796C mutants in the Multicloning Site (MCS) were digested with EcoRI and BamHI and the cDNA restriction enzyme result was analyzed by gel electrophoresis.

Plasma Membrane Expression of DDR1



	Sample Name	Subset	Count
	HEK-293 Secondary-ab	DDR1-	26419
	HEK-293 DDR1 WT	DDR1+	23727
	HEK-293 DDR1 N211Q	DDR1+	51082
	HEK-293 DDR1 Y796C	DDR1+	15423

Figure 5. *Plasma Membrane Expression of DDR1*. Flow cytometry analysis of DDR1-positive HEK cells shows that the DDR1 mutants N211Q and Y796C are expressed on the plasma membrane like the wild-type DDR1.

DDR1 Ligand Independent Activation and Downstream Signaling

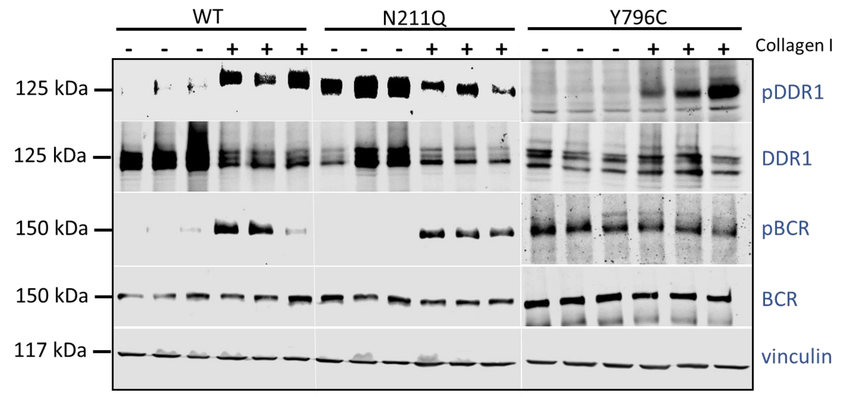


Figure 6. *DDR1 Activation and Signaling*. The western blot shows that DDR1 N211Q mutant undergo autophosphorylation in the absence of collagen compared to DDR1 WT and Y796P mutant. However, pBCR downstream signaling is only activated when collagen I is present. Interestingly, Y796C DDR1 mutant shows ligand independent downstream signaling.

pDDR1 and pBCR Expression

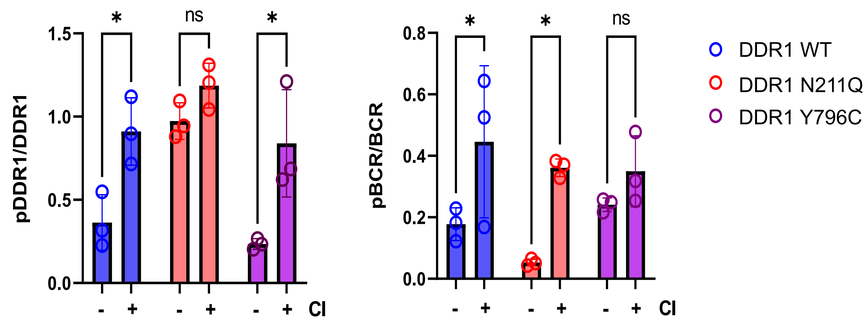


Figure 7. *Western blot quantification*. The activation of DDR1 and BCR are represented as the ratio of pDDR1 to total DDR1 and pBCR to total BCR, respectively. The graph represents the mean ± SD of three replicates within one experiment. (ns = not significant, * $p < 0.05$)

Conclusions

- The glycosylation site Asn211 in the DS-like domain acts as a negative regulator of DDR1 activation.
- Asn211Gln mutation results in ligand independent activation of DDR1, however the mutation does not seem to promote downstream signaling.
- Tyr796Cys mutation does not seem to regulate DDR1 ligand independent activation but initiates its downstream signaling.

Future Directions

Generate stable transfected WT and mutant DDR1 HEK-293 cells and conduct further analysis of DDR1 activation and BCR signaling.

References

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Funding & Acknowledgments

Berea/Aspirnaut™/Hal Moses Summer Research Internships; Vanderbilt University Medical Center; Center for Matrix Biology; Aspirnaut™; and Pozzi Lab Grants. R01-DK119212 (AP), 1I01BX002025

I would personally like to thank Dr. Billy and Dr. Julie Hudson, and the entire Aspirnaut team, for “discovering” me and my potential to thrive in the Aspirnaut Program this summer. I have been blessed with an amazing opportunity to cherish for life.

I greatly thank my research mentor, Dr. Gema Bolas, my lab PI, Dr. Ambra Pozzi, and colleagues of the Pozzi lab for welcoming me back this summer. My learning and understanding of the biomedical sciences has grown exponentially, and I have been blessed to be a part of an amazing team.

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