Designing Selective Inhibitors for Matrix Metalloproteinases

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Motivation

- Matrix metalloproteinases (MMPs) have been implicated in angiogenesis, invasion, and metastasis[1]
- MMP-9, especially, is involved in carcinogenesis and inflammation[1,2]
- Previous attempts at inhibition → side effects (nonspecific inhibition)[3]

The Approach

Designing the Inhibitors

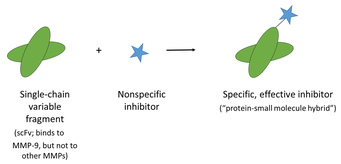
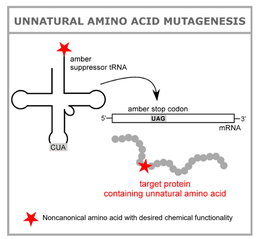


Figure 1 - The scheme for design of a specific MMP-9 inhibitor

- Connecting scFv and inhibitory small molecule using a bioorthogonal reaction
  - Introducing novel functional group into scFv using amber suppression (Fig. 2)

Figure 2 - Amber suppression integrates a noncanonical amino acid into a protein at the amber stop codon (adapted from RajBhandary)



Evaluating the Inhibitors

- Using yeast display (a technique in which yeast display on their surface a protein of interest)
  - Can detect binding of MMP to antibody
  - Can detect reaction of selected chemical moieties
- Using flow cytometry to measure extent of binding/reaction
- Can determine amount of protein displayed using epitope tags (see Fig. 3)

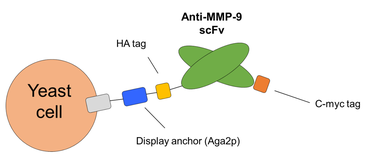
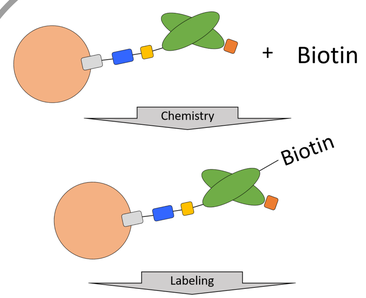


Figure 3 - Yeast display construct

Major aims

1. Identify chemistry to connect scFv and inhibitory small molecule
2. Identify scFv that will bind MMP-9, and evaluate its affinity for MMP-9



Fluorescent detection (flow cytometry)

Figure 4 - General strategy for evaluating bioorthogonal reactions on the yeast surface

Oxime Ligations

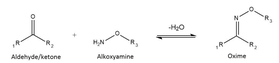


Figure 5—The oxime ligation reaction

Oxime Ligations under Different Conditions

Figure 6 - Oxime ligations were performed between yeast displaying ketone-containing antibodies or wild-type antibodies and biotin-alkoxyamine. Biotin was labeled and detected fluorescently using flow cytometry, and this graph shows the fluorescence of cells displaying the antibody on their surface.

Discussion

- The fluorescence of cells displaying the ketone-containing antibody and the fluorescence of those displaying the wild-type antibody was high compared to baseline fluorescence (red)
  - This reaction is not specific enough to serve our purposes, so oxime ligations were not pursued further
  - Nonspecific reaction is likely due to the presence of aldoses and ketoses on the surface of yeast cells

1. Bioorthogonal Reaction on the Yeast Surface

- Need a robust way to connect scFv to inhibitory small molecule
  - Reaction must be specific (should only occur at the scFv, not on other parts of the yeast surface)
- Considered oxime ligations and copper-catalyzed azide-alkyne cycloadditions ("click chemistry")

Click Chemistry

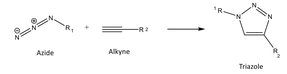


Figure 7—The click chemistry reaction

Click Reaction of Wild-Type and Azide-Containing scFvs

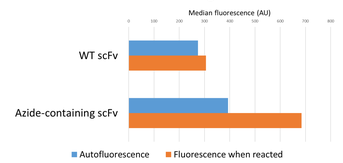
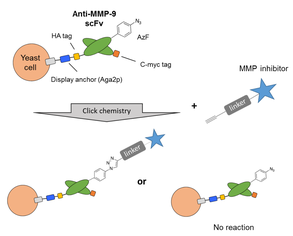


Figure 8 - The bioorthogonality of click chemistry on the yeast surface

Discussion

- This reaction is highly bioorthogonal (Fig. 8)
- 6-heptynoic acid, 10-undecynoic acid, and CUDC-101 reacted almost completely (Fig. 10)
- N-hydroxy 10-undecynamide reacted very little (Fig. 10)

Step 1: Reaction with inhibitory small molecule-alkyne



Step 2: Reaction with excess biotin-alkyne (fluorescently detectable)

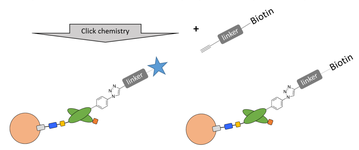


Figure 9 - The scheme for determining the extent of reaction of scFv with a non-detectable molecule. The greater the extent of reaction with inhibitor-alkyne, the lower the expected fluorescence

Click Chemistry with Inhibitory Small Molecules

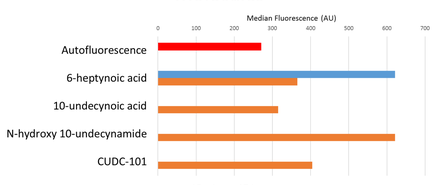


Figure 10 - Reaction of inhibitor-alkynes with azide-containing scFv

2. Evaluating scFv affinity for MMP-9

Three types of yeast, each displaying one purported MMP-9 binding protein (M0076, M0078, or DX-2802), were engineered, and treated with MMP-9 to see the extent of binding (labeling was performed against MMP-9).

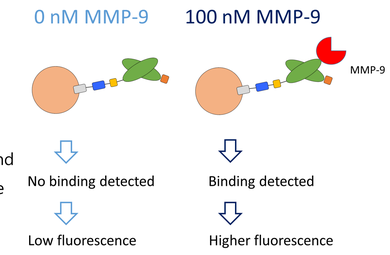


Figure 11 - Expected results of binding assay

Median Extent of Binding of MMP-9 to scFv

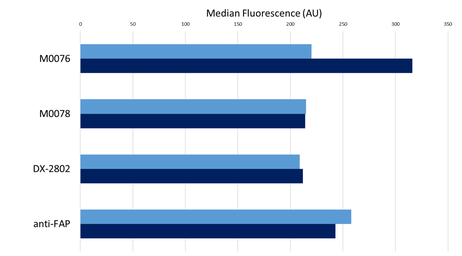


Figure 12 - Binding assay results. Anti-FAP was included as a negative control.

Discussion

- M0076 seems to have a high affinity for MMP-9
- M0078 and DX-2802 do not seem to bind to MMP-9 with any great affinity
  - The MMP-9 used may not have been in its active form

Current and Future Work

- Optimizing click chemistry conditions so all inhibitor-alkynes react extensively with azide-containing scFv
- Determining robust method for activating MMP-9
- Constructing inhibitors and evaluating their effectiveness and specificity for MMP-9

Acknowledgements

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References

[1] Rundhaug JE: Matrix metalloproteinases and angiogenesis. J Cell Mol Med 9: 267–285, 2005.
[2] Hu, P.E. Van den Steen, Q.X. Sang, G. Opdenakker. Matrix metalloproteinase inhibitors as therapy for inflammatory and vascular diseases. Nat. Rev. Drug Discov., 6 (6) (2007), pp. 480–498.
[3] Coussens, L. M., Fingleton, B. & Matrisian, L. M. Matrix metalloproteinase inhibitors and cancer: trials and tribulations. *Science* **295**, 2387–2392 (2002).

Image: RajBhandary U., Dept. of Biology, Mass. Inst. Of Technology. Cambridge, Mass.