Development of YEATS2 Inhibitor as a Potential Non-Small Cell Lung Cancer Cure

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Introduction

YEATS domain is a newly appreciated domain that is highly conserved across eukaryotes[1]. Researches have shown that human YEATS domain family members are strongly correlated with cancer proliferation by chromatin modification. For instance, the YEATS2 (YEATS domain containg 2) protein is responsible for the regulation of non-small cell lung cancer tumorigenesis[2]. By binding with H3K27ac, it regulates the acetylation level of H3K14ac, which in turns recruits the ATAC complex, and allows the chromatin to maintan an open structure for gene expression.

YEATS domain in general binds with acetylated (Kac) and crotonylated (Kcr) lysine via π-π-π stacking[3]. Specifically, a study regarding the interaction between YEATS2 and H3K27cr(1-34) has revealed that the W282 and Y262 residues in YEATS2 protein are responsible for the formation of the aromatic cage for the binding of lysine PTM (Kac/Kcr)[4]. (Fig. 1) It has also showed that by replacing the lysine PTM from Kac to Kcr, the binding affinity increases by 7 folds (IC_{50} = 196μM; determined by my colleague, Liu Sha).

Thus, our lab has hypothesized that the replacement of the lysine modification with other π-containing system could further enhance the binding affinity, and potentially create a peptide based competitive inhibitor. Prior to my research, our lab has already screened a number of modifications and discovered that YEATS2 protein is more selective towards benzoylated lysine (IC_{50} = 100μM) than crotonylated lysine. My work was to extend the screening in search of a better modification and sequence for the inhibitor.

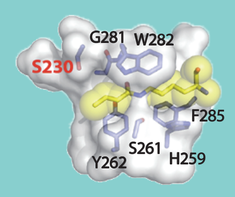

Fig. 1 Space filling model of the complex between YEATS2 (white) and crotonyl group of H3K27cr (yellow)[1]

Methodology

Modification Screening

To investigate the selectivity of YEATS2 protein towards different lysine modifications, we have to prepare peptides with the same sequence but with different modifications.

18 lysine modifications (M1-M18; where M1-M17 contain π systems and M18 = cyclopentanoylation) of the sequence H3K27 (22-32) were synthesized by my colleague Jiang Yixiang. They were screened using competitve photo-crosslinking assay, which will be elabotrated later.

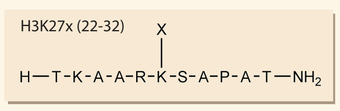

Fig. 2 The sequence of the H3K27x where X is the tested lysine modification

Sequence Screening

Other than the modification of lysine, the sequence of the peptide also contributes significantly to the binding affinity via interactions with surrounding residues. 11 peptides with different sequences were synthesized. These sequences are either fragments of the Histone H3 or Histone H2B protein, that are common PTM locations, and sites that were reported to have interaction with other YEATS domain-containing protein (AF9, ENL etc.)[5,6].

Quantification of Inhibitory Activity

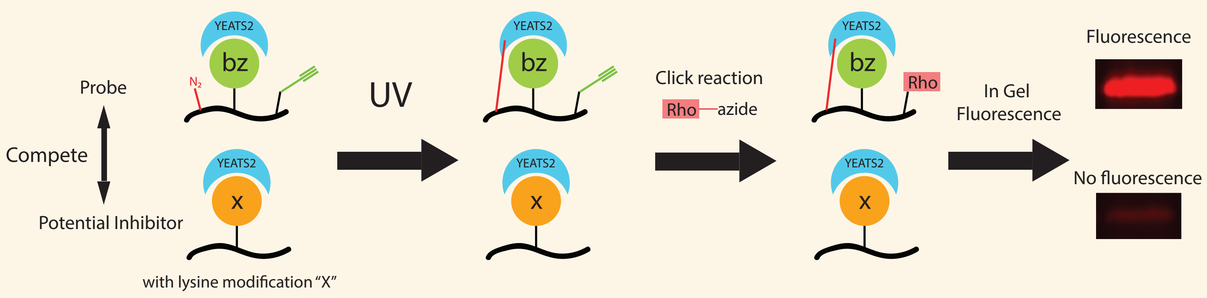

Fig 3. Schematic diagram illustrating the steps of competitve photo cross linking assay
A strong inhibitory activity will be reflected by a lack of fluoresecence in the protein band

The inhibitory activity of the potential peptide-based inhibitor was quantified by a competitive photo-crosslinking assay. (Fig. 3) Different concentrations of the potential inhibitor were prepared (0 - 1000μM). They were incubated with YEATS2 protein and a diazirine-based photo-crosslinking probe[7]. The probe has 4 characteristics (Fig. 4):

1. The sequence of H3(22-32), the natural binding site with YEATS2 protein
2. A benzoylated lysine group which has enhanced binding affinity with YEATS domain
3. A diazirine group for photo-crosslinking with the bound protein
4. An alkyne handle for the attachment of fluorescence group (Rhodamine)

After the incubation, the mixture was irradiated under UV to let the diazirine form a covalent bond with the bound YEATS2 protein. And rhodamine-azide, a fluorescent tag, was attached to the alkyne handle using click reaction. The YEATS2 protein-probe complex was then isolated using SDS-PAGE, followed by in-gel fluorescent visualization. If the inhibitor has a high activity, the probe will be inhibited from binding with the YEATS2 protein, generating no fluorescence in the protein band. The IC_{50} of the inhibitor was then determined from the fluorescent image using image analyzing tool.

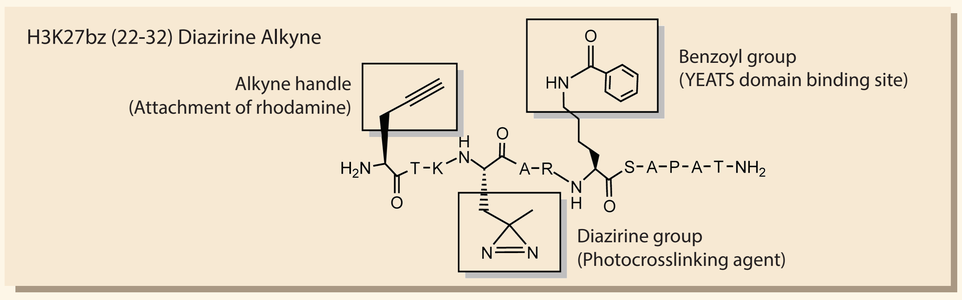

Fig. 4 Chemical structure of the diazirine-based probe

Screening Result

Modification Result

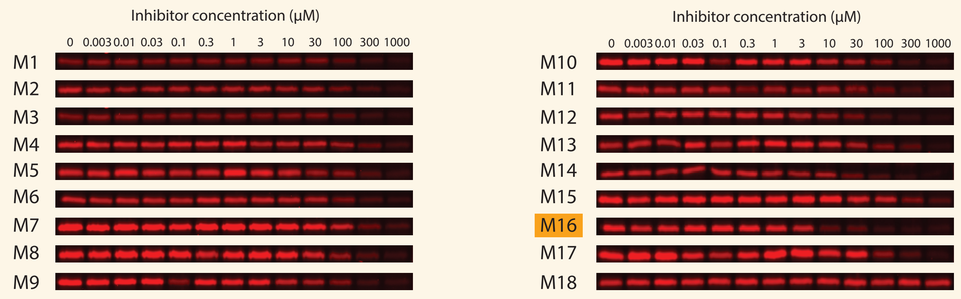

Fig 5. In-gel fluorescent image of the modification screening result

The above figure (Fig. 5) showed the in-gel fluorescent images of the 18 modifications. For the result of M16 (labeled in orange), we can clearly observe a disappearance in fluorescence intensity at 10μM in inhibitor concentration. We concluded that the M16 has the highest activity. The IC_{50} was determined to be 6.84 (± 1.76) μM. (Fig. 6B)

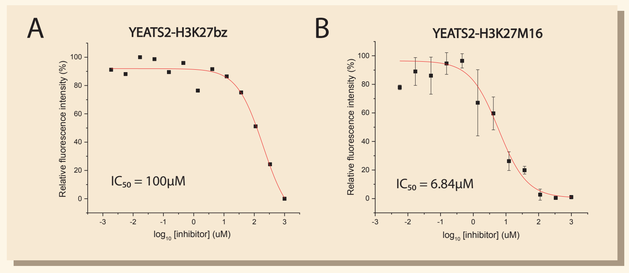

Fig. 6 Plot of relative fluorescence intensity against (A) log_{10}[H3K27bz] and (B) log_{10}[H3K27M16]. A 14-fold decrease in IC_{50} was observed.

Sequence Result

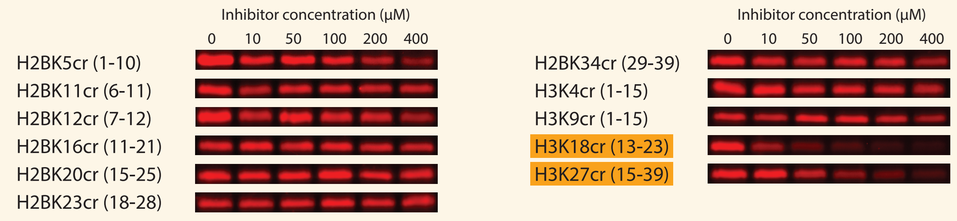

Fig 7. In-gel fluorescent image of the sequence screening result

The above figure showed the in-gel fluorescent images of the 11 peptide sequences. From the result, both H3K18cr (13-23) and H3K27cr (15-39) (labeled in orange) had relatively higher binding affinities with YEATS2 protein.

Sequence of the active peptides:

H3K18cr (13-23)	GKAPRKQLATK
H3K27cr (15-39)	APRKQLATKAARKSAPATGGVKPH

Where K is crotonyl lysine (Kcr)

Combining the Results

Base on the screening results, we identified M16 as the best lysine modification, and both sequences of H3K18cr and H3K27cr have decent binding activity with YEATS2 protein. Our next step was to determine the activity of the compound with the 2 combined variables, i.e. H3K18M16 and H3K27M16.

While the activity of H3K27M16 was already quantified in the first screening (IC_{50} = 6.84 ± 1.76) (Fig. 6B), the activity of H3K18Benfu was unknown. Thus, H3K18M16 was synthesized and its IC_{50} was determined to be 7.5 ± 2.7 μM. (Fig. 8)

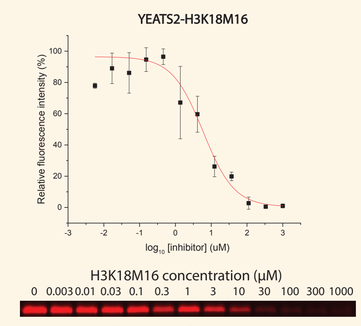

Fig 8. Plot of relative fluorescence intensity against log_{10} [H3K18M16] and the corresponding in-gel fluorescent image

Further Investigation

The potential inhibitor H3K27M16 and H3K18M16 could be further improved in various ways:

1. Optimizing the length of the peptide, which would enhance cell permeability.
2. Optimizing the amino acid on each position

If a decent inhibitory activity is achieved, it could be tested using endogenous YEATS2 protein and ultimately the non-small cell lung cancer cell line.

Reference

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