Developing Probes to Target c-Myc i-Motif DNA?

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What is i-Motif DNA?
- Formed by C-rich sequences
- Two parallel duplexes intercalated in an anti-parallel orientation
- Stabilised by hemiprotonated C-C+ base pairs

Evidence suggests regulate gene expression
- Potential to be powerful therapeutic targets

Aims
- Identify c-Myc i-motif binding peptides
- Identify c-Myc i-motif small molecules

Why Target the c-Myc i-Motif?
- c-Myc is a master regulator controlling many aspects of cell growth and metabolism
- Expression is tightly controlled by multiple mechanisms
- Frequently dysregulated in cancer but challenging to target

The regulation element NHE III controls 80-90% c-Myc transcription
- NHE III forms noncanonical DNA structures that likely regulate transcription

References

Phage Display: Identifying Peptides

M13 Phage Library
Peptides displayed on phage surface

Biotinylated i-Motif DNA
Biotin will bind to the streptavidin plate

1. Binding
Some peptides bind to the i-motif

2. Wash
Removal of unbound peptides

3. Elution
Collected i-motif binding peptides

4. Amplification
c-Myc binding peptides amplified in phage by E.coli

Panning
3-5 Cycles

Phage Display Results
- 1x10^7 12-mer peptides and 1x10^7 7-mer cyclic peptides were screened
- Five 12-mer peptides identified as binders
- No 7-mer cyclic peptide was identified
- 80% of sequences Pep5, suggesting it is the best peptide probe
- Circular dichroism determined Pep5 is a random coil

FID Results
- 1595 NCI compounds screened
- 22 compounds initially identified as binders
- 11 of these bound B-DNA and a further 8 were deemed poor probes
- Identified three promising c-Myc i-motif small molecules
- Next Steps: determine binding affinity and kinetics

Fluorescence Indicator Assay (FID): Identifying Small Molecules

Thiazole orange (TO)
doesn’t fluorescence

TO interacts with DNA and fluoresces
Small molecule displaces TO and fluorescence decreases