学位論文の要約

題目　Chemical biology studies on nucleic acid recognition, modification, and secondary structures

（核酸の認識と修飾とその２次元構造のケミカルバイオロジー研究）

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序論

Nucleic acids are the fundamental molecules that contain the blueprint of every living organism on Earth. The two main classes of nucleic acids deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) are present in the cells and constitute all life forms. DNA is the genetic material in most living organisms where RNA plays as genetic material in certain viruses and also plays important role in many cellular functions. Synthetic ligands capable of recognizing the specific DNA sequences are believed to be promising in targeted disruption of transcription factor-DNA interaction. Pyrrole-imidazole polyamides (PIP) are a class of small DNA binders that can be designed to target any destined DNA sequence. Herein we designed a cyclic PIP, which can target specific base pairs of the GLI1 binding site to inhibit the hedgehog pathway and the designed cyclic PIP suppressed the cancer stem cell population along with temozolomide. Modifications on nucleic acids s were found to regulate various cellular processes and detection of modification is a key step in delineating its role. Here, we demonstrate direct RNA nanopore sequencing to detect RNA modifications on long RNAs at the single-molecule level. Using a selective chemical probe towards RNA modification we have mapped the inosine and pseudouridine modification in synthetic RNA and mouse transcriptome.

Nucleic acid Recognition

Pyrrole-imidazole polyamides (PIP) are best characterized programmable synthetic molecules that can be programmed to bind to any predetermined DNA sequence. Synthetic transcription factors are attractive in the field of molecular medicine since they can mimic the function of natural transcription factors and regulate the expression of specific genes. Although oligonucleotides can recognize specific sequences with high affinity and modulate the function of specific genes it is limited by poor cellular uptake. taking advantage of sequence selective binding and transgene-free uptake in cells various PIP has been designed and evaluated to inhibit the eukaryotic transcription. Cancer stem cells (CSCs) are a small subpopulation of cells within the cancer tissue that play major roles in metastasis, drug resistance, and recurrence. We designed a sequence-specific cyclic PIP capable of targeting Gli-mediated transcription and inhibiting the hedgehog pathway which is implied to play a major role in cancer stem cell proliferation. The DNA binding affinities of cyclic polyamides were superior to corresponding hairpin polyamides which were widely used for DNA recognition and inhibition earlier. Mechanistically, the cyclic PIPs blocked the Gli function and combinatorial treatment of cyclic PIPs and temozolomide (TMZ) to glioblastoma and brain cancer stem cells showed increased cell death compared to TMZ alone. Taken together, cyclic PIPs targeting Gli-mediated transcription can be a promising strategy in suppressing the CSCs.

Detection of RNA modifications using Nanopore Sequencing

Apart from the conventional five primary or canonical bases which form genetic code, DNA and RNA bases also get modified and form non-canonical bases. Modification in DNA and RNA play a vital role in many functions and controls gene expression and its regulation. The RNA modification opened up a new field epitranscriptomics and to date, more than 170 different types of modifications are identified in various coding and non-coding RNAs. In the earlier days, RNA modifications are identified using thin-layer chromatography, HPLC, and mass spectrometry. But these methods have very low sensitivity and can be applied only to highly abundant modifications. With the advent of next-generation sequencing (NGS) it is now possible to sequence a multitude of samples with low cost and very low error rate. Second-generation sequencing such as Illumina, ion torrent can aid in the identification of RNA modifications. But the current methods to identify RNA modifications with short-read sequencing are laborious and direct RNA sequencing by Nanopore sequencing is a viable alternative. Herein, we harnessed the selective reactivity of the acrylonitrile towards the Inosine (I) and pseudouridine (Ψ) modifications and developed a chemical probe-based direct RNA sequencing method by artificially inducing signature error profiles. Using our chemical probe, we

selective assessed I and Ψ RNA modifications in both in vitro synthesized RNA and mouse brain without the need for a null dataset using knockouts. We also devised Nano ICE-Seq, a protocol to overcome the low coverage issue associated with direct RNA sequencing. Furthermore, we developed a multi-parameter analysis strategy and performed a proof-of-concept study to identify multiple RNA modifications on the mouse transcriptome. Our chemical biology approach may facilitate the knockout-free detection of RNA modification to identify disease-associated epitranscriptome markers in clinical scenarios.