盧煜明

DENNIS LO

02 MOLECULAR DIAGNOSTICS

Circulating Fetal Nucleic Acids

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Research Progress Summary

Led by Dennis Lo and Rossa Chiu, the project team has been pursuing a novel facet of cell-free DNA biology, which the team termed as fragmentomics. This refers to the analysis of the ending patterns and fragmentation characteristics of cell-free DNA molecules. This research area has attracted little attention in the past. Cell-free DNA molecules exist in circulation in a naturally fragmented form. By studying the end sequences of cell-free DNA molecules systematically and across the whole genome, the team initially made the surprising observation that cell-free DNA fragmentation is a non-random process. There are genomic locations which featured more regularly at cell-free DNA ends than others which have been termed as the preferred ends. Those findings then prompted the team to investigate the ends of cell-free DNA from various angles. The team found certain motif sequences to be overrepresented among cell-free DNA molecules and then showed that the ends may be blunt or jagged in nature in both plasma and urine. Those intriguing observations spurred the team to study the biological basis by exploring the roles of various DNA digesting enzymes, including Deoxyribonuclease 1 Like 3 (DNase1L3), Deoxyribonuclease I (DNase1) and DNA Fragmentation Factor Subunit Beta (Dffb). During this journey, the team uncovered pathologies with altered activity levels of those enzymes that would be associated with perturbations in the cell-free DNA profiles. In other words, by improving their understanding of biological mechanisms, one may ultimately find novel ways to develop diagnostic tests. One of the team's research publications on fragmentomics (Han et al. *Genome Res* 2021; 31: 2008-2021) has been featured on the cover of the journal, *Genome Research*. Interestingly, since the research team began the exploration of fragmentomics, interests from other groups on the topic have also gathered momentum. In other words, the team's academic pursuit has led to the emergence of a new research field and the journal, *Science*, even has invited the team to contribute a review on the topic (Lo et al. *Science* 2021; 372: eaaw3616).

On the other hand, the team has been actively harnessing the power of applying single molecule sequencing to the analysis of cell-free DNA. First, the team made the surprising observation that there was an under-appreciated population of long cell-free DNA molecules in maternal plasma. This class of cell-free DNA molecules enabled new and additional information to be gathered about pregnancy health and fetal genetic profile as shown by the work in Yu et al. (*Proceedings of the National Academy of Sciences U S A* 2021; 118: e2114937118). Second, cytosine methylation of CpG dinucleotides is one important strategy to distinguish tissue origin of cell-free DNA molecules or one that allows the detection of pathological processes associated with aberrant methylation profile via cell-free DNA analysis. Bisulfite sequencing has remained as the key technology to study DNA methylation. However, bisulfite converts all unmethylated cytosines into thymine residues, even when the cytosines are out of the context of CpG sites. Consequently, bisulfite sequencing results in reduced sequence resolution and adds ambiguity. To resolve this, the team has attained a major achievement by successfully developing an approach based on studying the kinetic features of single molecule sequencing data to distinguish methylated cytosine without the need for bisulfite treatment. The team believes this method would greatly enhance and facilitate any genetic and genomic studies focused on DNA methylation. Those data have been published in Tse et al. *Proceedings of the National Academy of Sciences* USA 2021; 118: e2019768118. Since the release of the publication, they have been approached by a dozen of research groups to provide the bioinformatics code to enable their adoption of the published methodology.

Research and Scholarship

Research Awards and Recognitions

Member's Name	Details	
	Award	Organisation
Dennis Lo	The ESHG Mendel Award 2021	European Society of Human Genetics (ESHG)
	Breakthrough Prize in Life Sciences	Breakthrough Prize
	Royal Medal - Biological Sciences	Royal Society
Dennis Lo Rossa Chiu Allen Chan	Top 20 Translational Researchers of 2020	Nature Biotechnology

Academic Editorship

Member's Name	Details	
	Role	Journal
Dennis Lo	Associate Editor	Clinical Chemistry
		Genomic Medicine
	Senior Editor	eLife
	Editorial Board member	Cancer Communications
		Journal of Pathology
		Philosophical Transactions of the Royal Society B
		Disease Markers
		Prenatal Diagnosis
		Journal of Genomes and Exomes
		Marrow
		American Journal of Hematology
Rossa Chiu	Associate Editor	Clinical Chemistry
		Human Genetics and Genomics Advances
	Editorial Board member	Clinical Biochemistry
		Critical Reviews in Clinical Laboratory Sciences
		The Clinical Biochemist Reviews

Grants and Consultancy

Name	Project Title	Funding Source	Start Date (dd/mm/yyyy)	End Date (dd/mm/yyyy)	Amount (HK$)
Dennis Lo	Centre for Novostics	Innovation and Technology Commission – InnoHK Scheme	01/05/2020	30/04/2025	Not to be disclosed
	Genomic Approaches for Predicting Severity of Organ Dysfunction and Outcomes in Sepsis: A Prospective Cohort Study in Adult Critically Ill Patients with Sepsis	The Chinese University of Hong Kong Faculty of Medicine – Faculty Innovation Award	01/01/2020	31/12/2022	750,000
Dennis Lo	Plasma DNA as a Platform Technology for Cancer Detection	Research Grants Council – Theme-based Research Scheme	01/12/2016	30/11/2021	28,570,000
Rossa Chiu	Plasma DNA as a Platform Technology for Cancer Detection	The Chinese University of Hong Kong – Focused Innovations Scheme C	01/12/2016	30/11/2021	2,093,500

Publications

A. Journal Papers

1. Lo YMD, Han DSC, Jiang P, Chiu RWK. Epigenetics, fragmentomics, and topology of cell-free DNA in liquid biopsies. *Science*. 2021;372(6538). doi:10.1126/science.aaw3616. (Review)

2. Chiu RWK, Lo YMD. Cell-free fetal DNA coming in all sizes and shapes. *Prenatal Diagnosis*. 2021;41(10):1193-1201. doi:10.1002/pd.5952. (Review)

3. Tse OYO, Jiang P, Cheng SH, Peng W, Shang H, Wong J, Chan SL, Poon LCY, Leung TY, Chan KCA, Chiu RWK, Lo YMD. Genome-wide detection of cytosine methylation by single molecule real-time sequencing. *Proceedings of the National Academy of Sciences*. 2021;118(5):e2019768118. doi:10.1073/pnas.2019768118.

4. Zhou Z, Cheng SH, Ding SC, Heung MMS, Xie T, Cheng THT, Lam WKJ, Peng W, Teoh JYC, Chiu PKF, Ng C-F, Jiang P, Chan KCA, Chiu RWK, Lo YMD. Jagged ends of urinary cell-free DNA: Characterization and feasibility assessment in bladder cancer detection. *Clinical Chemistry*. 2021;67(4):621-630. doi:10.1093/clinchem/hvaa325.

5. Sin STK, Ji L, Deng J, Jiang P, Cheng SH, Heung MMS, Lau CSL, Leung TY, Chan KCA, Chiu RWK, Lo YMD. Characteristics of fetal extrachromosomal circular DNA in maternal plasma: Methylation status and clearance. *Clinical Chemistry*. 2021;67(5):788-796. doi:10.1093/clinchem/hvaa326.

6. Han DSC, Lo YMD. The nexus of cfDNA and nuclease biology. *Trends in Genetics*. 2021;37(8):758-770. doi:10.1016/j.tig.2021.04.005. (Review)

7. Danesi R, Lo YMD, Oellerich M, Beck J, Galbiati S, Re MD, Lianidou E, Neumaier M, van Schaik RHN. What do we need to obtain high quality circulating tumor DNA (ctDNA) for routine diagnostic test in oncology? – Considerations on pre-analytical aspects by the IFCC workgroup cfDNA. *Clinica Chimica Acta*. 2021;520:168-171. doi:10.1016/j.cca.2021.05.033.

8. Lo YMD. Noninvasive prenatal testing: Advancing through a virtuous circle of science, technology and clinical applications. *Prenatal Diagnosis*. 2021;41(10):1190-1192. doi:10.1002/pd.5978. (Commentary)

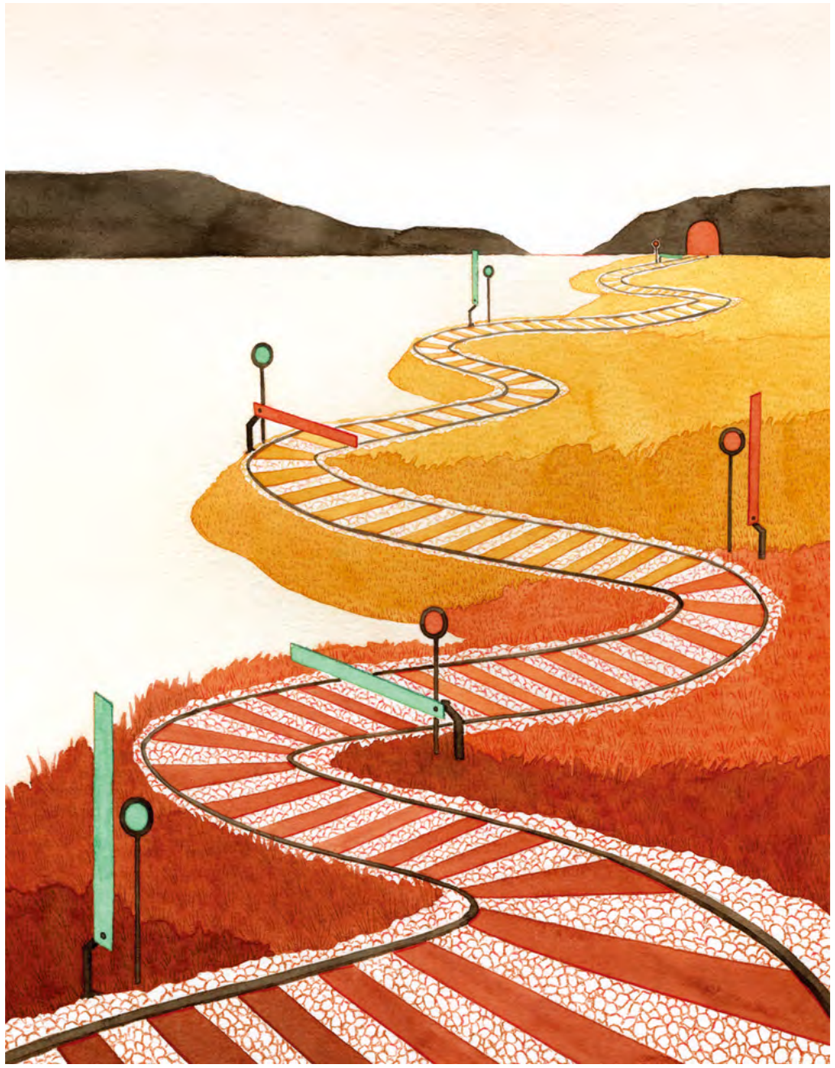
9. Ma ML, Yakovenko S, Zhang H, Cheng SH, Apryshko V, Zhavoronkov A, Jiang P, Chan KCA, Chiu RWK, Lo YMD. Fetal mitochondrial DNA in maternal plasma in surrogate pregnancies: Detection and topology. *Prenatal Diagnosis*. 2021;41(3):368-375. doi:10.1002/pd.5860.

10. Han DSC, Ni M, Chan RWY, Wong DKL, Hiraki LT, Volpi S, Jiang P, Lui KO, Chan KCA, Chiu RWK, Lo YMD. Nuclease deficiencies alter plasma cell-free DNA methylation profiles. *Genome Research*. 2021;31(11):2008-2021. doi:10.1101/gr.275426.121.

11. Yu SCY, Jiang P, Peng W, Cheng SH, Cheung YTT, Tse OYO, Shang H, Poon LC, Leung TY, Chan KCA, Chiu RWK, Lo YMD. Single-molecule sequencing reveals a large population of long cell-free DNA molecules in maternal plasma. *Proceedings of the National Academy of Sciences*. 2021;118(50):e2114937118. doi:10.1073/pnas.2114937118.

12. Gai W, Zhou Z, Agbor-Enoh S, Fan X, Lian S, Jiang P, Cheng SH, Wong J, Chan SL, Jang MK, Yang Y, Liang RH, Chan WK, Ma ES, Leung TY, Chiu RW, Valantine H, Chan KA, Lo YD. Applications of genetic-epigenetic tissue mapping for plasma DNA in prenatal testing, transplantation and oncology. *eLife*. 2021;10. doi:10.7554/elife.64356.

13. Vong JSL, Ji L, Heung MMS, Cheng SH, Wong J, Lai PBS, Wong VWS, Chan SL, Chan HLY, Jiang P, Chan KCA, Chiu RWK, Lo YMD. Single cell and plasma RNA sequencing for RNA liquid biopsy for hepatocellular carcinoma. *Clinical Chemistry*. 2021;67(11):1492-1502. doi:10.1093/clinchem/hvab116.



Cell-free DNA in plasma consists of fragments of DNA and has been used for noninvasive prenatal testing, cancer liquid biopsies, and transplantation monitoring. Han et al. demonstrate the interrelationships between nucleases, cell-free DNA fragmentation, and DNA methylation, which are depicted in this artistic illustration. The railroad (representing DNA) enters a red tunnel (representing a blood vessel) in the distant mountain. The railroad has red and green sign posts, denoting methylated cytosines and unmethylated cytosines on the DNA, respectively. Barriers of different colors are different nucleases, preferring to cleave (down position) or not cleave (up position) the DNA. DNASE1 (red barriers) prefers to cleave at unmethylated cytosines, while DNASE1L3 (green barriers) prefers to cleave at methylated cytosines. Thus, nuclease-mediated cell-free DNA fragmentation is informed by underlying DNA methylation. (Cover art using watercolor and colored pencils on paper by Carmen Ng [https://www.carmen-ng.com/], based on a concept from Dennis Lo.

Source: Cover illustration of journal issue publishing Han et al. Nuclease deficiencies alter plasma cell-free DNA methylation profiles. Genome Research 2021; 31: 2008-2021.