Personal note

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Short CV

2013-2018
Master of Science in Biomedical Science
Ghent University

2022
Visiting doctoral fellow
Salzman lab, Stanford

2019-2023
Doctoral fellow
OncoRNALab, Ghent University

Contact

mariekevromman@gmail.com

www.linkedin.com/in/marieke-vromman

https://twitter.com/MariekeVromman

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fwo

GHENT UNIVERSITY

Examination committee

prof. dr. Tim De Meyer, BIOBIX lab, Department of Data Analysis and Mathematical Modelling, Ghent University

prof. dr. ir. Katleen De Preter, Translational Oncogenomics and Bioinformatics Lab, Department of Biomolecular Medicine, Ghent University

prof. dr. Kaat Durinck, Pediatric Precision Oncology Lab, Department of Biomolecular Medicine, Ghent University

prof. dr. Celine Everaert, Translational Oncogenomics and Bioinformatics Lab, Department of Biomolecular Medicine, Ghent University

prof. dr. Jørgen Kjems, Department of Molecular Biology and Genetics - RNA Biology and Innovation, Aarhus University

dr. Joost Kluiver, Faculty of Medical Sciences, University of Groningen, Groningen

dr. Wim Trypsteen, HIV Cure Research Center, Department of Internal Medicine and Pediatrics, Ghent University

Supervisor

prof. dr. ir. Jo Vandesompele, OncoRNALab, Department of Biomolecular Medicine, Ghent University

Co-supervisor

prof. dr. ir. Pieter-Jan Volders, OncoRNALab, Department of Biomolecular Medicine, Ghent University

Doctoral advisory committee

prof. dr. ir. Pieter Mestdagh, OncoRNALab, Department of Biomolecular Medicine, Ghent University

prof. dr. ir. An Hendrix, Laboratory of Experimental Cancer Research, Department of Human Structure and Repair, Ghent University

OncoRNALab

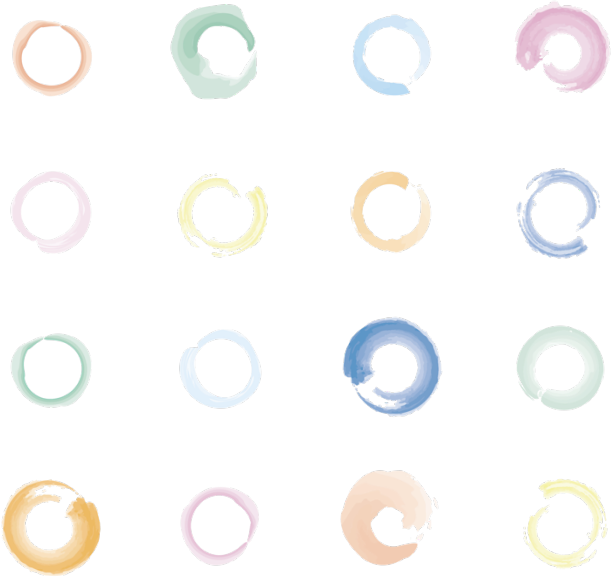
CRIG

GHENT UNIVERSITY

FACULTY OF MEDICINE AND HEALTH SCIENCES

Chasing circles: developing a toolbox for circRNA detection and validation

Marieke Vromman



Thesis is submitted as fulfillment of the requirements for the degree of Doctor in Health Sciences, 2022-2023.

Supervisor: prof. dr. ir. Jo Vandesompele
Co-supervisor: prof. dr. ir. Pieter-Jan Volders

OncoRNALab
Cancer Research Center Ghent

Department of Biomolecular Medicine
Faculty of Medicine and Health Sciences

Summary

Circular RNAs (circRNAs) are covalently closed RNA molecules present in a variety of species. They have been linked to multiple diseases, including cancer. For most circRNAs, a precise function and working mechanism are lacking. Due to their circular nature, circRNAs are more resistant to degradation and can be stably present in biofluids such as blood, making them attractive biomarker candidates.

In general, circRNAs are detected in RNA sequencing data with specific **circRNA detection tools**. This is then followed by **validation** using RNase R and RT-qPCR.

However, the field of circRNA research is relatively new and lacks established gold standards. To address these challenges, my doctoral project aimed to enhance circRNA research methodologies.

First, we reviewed existing circRNA databases, comparing their contents, annotations, and ease of use (*paper 1*).

Simultaneously, to address the diverse protocols for circRNA **validation**, we extensively tested and optimized circRNA primer design, RNase R treatment, and RT-qPCR methods (*paper 2* and *paper 3*).

Finally, we evaluated various **circRNA detection tools** to determine the most reliable option for circRNA detection in total RNA sequencing data (*paper 4)*. While tool-specific precision values were consistently high, the number of predicted circRNAs varied greatly among the tools. Based on our research findings, we drafted general guidelines for circRNA detection tool development, benchmarking, and circRNA validation. These guidelines aim to improve the reliability and reproducibility of circRNA research in the future.

In summary, this doctoral thesis critically evaluated current circRNA detection and validation methods and proposed strategies to enhance future circRNA research.

Samenvatting

Circulaire RNA's (circRNA's) zijn RNA-moleculen aanwezig in een mensen, dieren, en platen. Ze worden in verband gebracht met meerdere ziekten, waaronder kanker. Voor de meeste circRNA’s is er geen precieze functie en werkingsmechanisme bekend. Door hun circulaire structuur zijn circRNA's beter bestand tegen afbraak en kunnen ze stabiel aanwezig zijn in lichaamsvloeistoffen zoals bloed. Hierdoor zijn ze aantrekkelijke biomerker kandidaten.

Over het algemeen worden circRNA's gedetecteerd in RNA-sequencing experimenten met specifieke **circRNA-detectietools**. Vervolgens worden ze **gevalideerd** met behulp van RNase R en RT-qPCR.

Echter, het onderzoeksgebied van circRNA is relatief nieuw en mist gouden standaarden. Om deze uitdagingen aan te pakken, was het doel van mijn doctoraatonderzoek om circRNA-onderzoeksmethoden te verbeteren.

Ten eerste hebben we bestaande circRNA-databanken beoordeeld, waarbij we hun inhoud, annotaties en gebruiksgemak hebben vergeleken (*paper 1*).

Tegelijkertijd hebben we circRNA-primerontwerp, RNase R-behandeling en RT-qPCR-methoden geoptimaliseerd om de uiteenlopende protocollen voor circRNA-**validatie** aan te pakken (*paper 2* en *paper 3*).

Tot slot hebben we verschillende **circRNA-detectietools** geëvalueerd om de meest betrouwbare optie voor circRNA detectie in RNA-sequencing data te bepalen (*paper 4*). Hoewel de precisie van de tools consistent hoog was, varieerde het aantal voorspelde circRNA's sterk tussen de verschillende tools. Op basis van onze onderzoeksresultaten hebben we algemene richtlijnen opgesteld voor de ontwikkeling, benchmarking en validatie van circRNA-detectietools.

Samenvattend heeft dit proefschrift de huidige methoden voor circRNA-detectie en -validatie kritisch geëvalueerd en strategieën voorgesteld om toekomstig circRNA-onderzoek te verbeteren.

Publications included in this thesis

Vromman M, Vandesompele J and Volders PJ (2021) Closing the circle: current state and perspectives of circular RNA databases. Brief Bioinform (*paper 1*)

Vromman M, Yigit N, Verniers K, Lefever S, Vandesompele J and Volders PJ (2021) Validation of Circular RNAs Using RT-qPCR After Effective Removal of Linear RNAs by Ribonuclease R. Curr Protoc (*paper 2*)

Vromman M, Anckaert J, Vandesompele J and Volders PJ (2022) CIRCprimerXL: Convenient and High-Throughput PCR Primer Design for Circular RNA Quantification. Frontiers in Bioinformatics (*paper 3*)

Vromman M, Anckaert J, Bortoluzzi S, Buratin A, Chen CY, Chu Q, Chuang TJ, Dehghannasiri R, Dieterich C, Dong X, Flicek P, Gaffo E, Gu W, He C, Hoffmann S, Izuogu O, Jackson MS, Jakobi T, Lai EC, Nuytens J, Salzman J, Santibanez-Koref M, Stadler P, Thas O, Vanden Eynde E, Verniers K, Wen G, Westholm J, Yang L, Ye CY, Yigit N, Yuan GH, Zhang J, Zhao F, Vandesompele J, Volders PJ (2023) Large-scale benchmarking of circRNA detection tools reveals large differences in sensitivity but not in precision. Nature Methods (accepted in principle) (*paper 4*)

Other publications

Lorenzi L, Chiu HS, Avila Cobos F, Gross S, Volders PJ, Cannoodt R, Nuytens J, Vanderheyden K, Anckaert J, Lefever S, Tay AP, de Bony EJ, Trypsteen W, Gysens F, **Vromman M**, Goovaerts T, Birkballe Hansen T, Kuersten S, Nijs N, Taghon T, Vermaelen K, Bracke KB, Saeys Y, De Meyer T, Deshpande NP, Anande G, Chen T-W, Wilkins MR, Unnikrishnan A, De Preter K, Kjems J, Koster J, Schroth GP, Vandesompele J, Sumazin P & Mestdagh P (2021) The RNA Atlas expands the catalog of human non-coding RNAs. Nat Biotechnol

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