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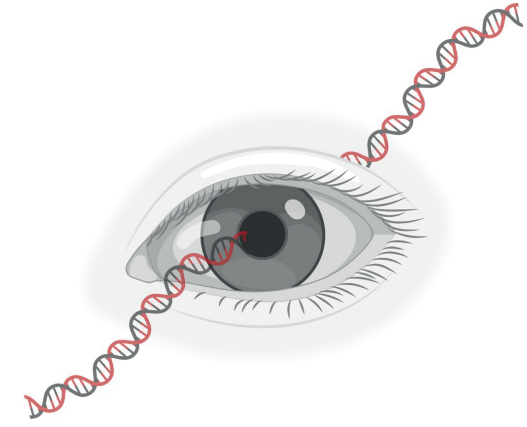
Unraveling the therapeutic potential of SAMMSON lncRNA inhibition in uveal melanoma



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De grote lijnen

Uveaal melanoom (UM) is een eerder zeldzaam, maar zeer dodelijke vorm van kanker. UM is de meest voorkomende intra-oculaire kwaadaardige ziekte bij volwassenen en momenteel bestaan er enkel behandelingen voor de primaire tumor. Ondanks deze behandeling, ontwikkelt bijna de helft van de patiënten uitzaaiingen, meestal in de lever, wat gewoonlijk fataal is binnen 1 tot 3 jaar. Dit toont aan dat er een hoge nood is aan de ontwikkeling van nieuwe behandelingen en dan voornamelijk voor metastatische UM.

In deze thesis heb ik mij gefocust op het lang niet-coderend RNA (lncRNA) SAMMSON dat tot expressie komt in (uveale) melanomen en afwezig is in gezonde weefsels. Door dit lncRNA te onderdrukken met antisense oligonucleotiden (ASOs), hebben we ervoor gezorgd dat de hoeveelheid aan SAMMSON vermindert, wat resulteert in het afsterven van deze UM cellen. Dit werd aangetoond in zowel UM cellijnen als proefdieren. Aangezien SAMMSON afwezig is in zo goed als alle gezonde humane weefsels, kan inhibitie van SAMMSON in zowel primaire als uitgezaaide kanker resulteren in tumor celdood zonder gezonde weefsels aan te tasten, wat de mogelijkheid voor een potentiële effectieve en veilige UM therapie aantoont.

Om het therapeutisch potentieel te verhogen werd een collectie van chemische verbindingen in combinatie met SAMMSON inhibitie getest. Daaruit werd mTOR inhibitor GDC-0349 geïdentificeerd als een chemische verbinding die het effect van SAMMSON inhibitie verder versterkt, wat resulteert in een toename van het aantal afstervende UM cellen. Dit effect werd eveneens geobserveerd in andere kankercellijnen en in combinatie met andere therapeutische moleculen zoals siRNAs.

The details

Due to its resistance to chemotherapy and disapointing results with existing immunotherapeutic agents, the unmet need for metastatic UM therapies remains high. Since the field of lncRNAs is emerging and the role of many lncRNAs in disease regulation has been demonstrated, we investigated the therapeutic potential in UM of a specific lncRNA called SAMMSON.

Pan-cancer RNA sequencing data revealed higher SAMMSON expression levels in metastatic tumors, compared to matching primary tumors and no expression in adult healthy tissues (paper 1). Through perturbation of SAMMSON in vitro and in vivo using LNA-modified gapmer ASOs, I demonstrated the importance of SAMMSON expression for UM cell survival. SAMMSON inhibition reduces UM cell viability through induction of apoptosis, irrespective of the mutational status of the UM cell line. Mechanistically, we could demonstrate SAMMSON to be involved in the regulation of mitochondrial and cytosolic translation.

To further improve the therapeutic effect, an in vitro screen was performed where SAMMSON inhibition was combined with a library of 2911 clinical stage compounds (paper 2) to identify synergizing compounds. Cell viability results revealed mTOR inhibitor GDC-0349 to be the most potent compound. By means of confocal microscopy, I was able to demonstrate that GDC-0349 enhances the cellular uptake and reduces the lysosomal accumulation of the SAMMSON ASO, which is suggestive for improved ASO activity. These observations could be confirmed with the enhanced SAMMSON knockdown observed in the combined treatment. Additionally, improved target knockdown in the combined treatment could also be demonstrated when using alternative mTOR inhibitors as well as additional lipid particle complexed and encapsulated ASOs and siRNAs in multiple cell lines.

Publications in this thesis

Paper 1

The long non-coding RNA SAMMSON is essential for uveal melanoma cell survival

Shanna Dewaele, Louis Delhaye, Boel De Paepe, Eric De Bony, Jilke De Wilde, Katrien Vanderheyden, Jasper Anckaert, Nurten Yigit, Justine Nuytens, Eveline Vanden Eynde, Joél Smet, Maxime Verschoore, Fariba Nemati, Didier Decaudin, Manuel Rodrigues, Peihua Zhao, Aart Jochemsen, Eleonora Leucci, Jo Vandesompele, Jo Van Dorpe, Jean-Christophe Marine, Rudy Van Coster, Sven Eyckerman and Pieter Mestdagh.
Oncogene. 2021

Paper 2

mTOR inhibition enhances delivery and activity of antisense oligonucleotides in uveal melanoma cells

Shanna Dewaele, Louis Delhaye, Boel De Paepe, Bram Bogaert, Ramiro Martinez, Jasper Anckaert, Nurten Yigit, Justine Nuytens, Rudy Van Coster, Sven Eyckerman, Koen Raemdonck and Pieter Mestdagh
Under review

Submitted patents

Combinations of therapeutic agents for treating uveal melanoma
submission number: EP19177294.6; PCT: PCT/E P2020/064430, 2020

mTOR inhibition improves lipid mediated antisense oligonucleotide and small interfering RNA delivery and activity
Submission number: EP21197163.5; PCT: PCT/E P2021/004; 2021

Full text thesis