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Heparan sulfate proteoglycans: key moderators of the interaction of multiple myeloma with the bone marrow niche

Ren, Z.

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CHAPTER

7

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Nederlandse samenvatting

Multipel myeloom (MM) is een veelal ongeneeslijke hematologische maligniteit die wordt gekenmerkt door de klonale expansie van kwaadaardige plasmacellen in het beenmerg (BM). De overleving en proliferatie van myeloomcellen in het beenmerg is sterk afhankelijk van signalen afkomstig uit de micro-omgeving.

In veel gevallen van multipel myeloom is buitensporige activatie van Wnt/ β -catenine signalering een belangrijke oorzaak van proliferatie en geassocieerd met ziekteprogressie. Omdat activerende mutaties in deze signaleringsroute zeer zelden voorkomen bij myeloompatiënten, suggereert dit dat Wnt-signalering in MM gedreven wordt door autocriene en/of paracriene Wnt-liganden. In **hoofdstuk 2** beschrijven we de tot dusver onbekende rol van R-spondin/LGR4 signalering bij de activatie van de Wnt-signaleringsroute in MM. We hebben laten zien dat LGR4 tot expressie komt in primaire myeloomcellen en in humane MM-cellijnen, maar niet in gezonde plasmacellen of B-cellen in een eerder ontwikkelingsstadium. De overmatige expressie van LGR4 in MM-cellen wordt transcriptioneel gereguleerd door IL-6/STAT3-signalering. De expressie van LGR4 biedt myeloomcellen de mogelijkheid door (pre-)osteoblasten geproduceerde R-spondins aan zich te binden. Dit zorgt vervolgens voor stabilisatie van de Wnt-receptor, resulterend in sterk verhoogde gevoeligheid voor autocriene en paracriene Wnt-liganden. Daarnaast laten we zien dat remming van Wnt/ β -catenine signalering door het blokkeren van β -catenine gemedieerde transcriptie, het remmen van autocriene Wnt secretie of het verlagen van LGR4 expressie de deling van myeloomcellen in zeer sterke mate vermindert. Hieruit concluderen we dat R-spondin/LGR4 signalering sterk betrokken is bij de activatie van de Wnt-signaleringsroute in MM. Deze resultaten pleiten voor LGR4 als een potentieel therapeutisch target voor de behandeling van MM.

Hoge expressie van syndecan-1 is een belangrijk kenmerk van zowel gezonde plasmacellen als van kwaadaardige myeloomcellen. In **hoofdstuk 3** laten we voor het eerst zien dat humane myeloomcellijnen constitutieve activatie van de Wnt/ β -catenine signaleringsroute vertonen en dat remming hiervan, door het blokkeren van β -catenine gemedieerde transcriptie of onderdrukken van autocriene Wnt secretie, resulteert in verminderde celgroei. We demonstreren dat zowel humane myeloomcellijnen als primaire myeloomcellen in hoge mate heparaansulfaten (HS) tot expressie brengen op de celmembraan. Vervolgens laten we zien dat CRISPR/Cas9-gemedieerde knock-out van EXT1 resulteert in volledig verlies van syndecan-1 HS op het celoppervlak. In afwezigheid van heparaansulfaten laten myeloomcellen sterk verminderde autocriene Wnt/ β -catenine signalering zien en verminderde expressie van door Wnt gereguleerde genen. Daarnaast resulteerde induceerbare knockdown van EXT1 in een significante vermindering van de MM celgroei. Belangrijk is dat de verminderde groei als gevolg van HS-deficiëntie niet hersteld kon worden door de myeloomcellen te kweken samen met stromale cellen uit het beenmerg

(BMSCs). Vervolgens laten we zien dat verlies van HS ook de activatie van Wnt-signalering door paracriene Wnt-liganden vermindert. In lijn met hoofdstuk 2 laten we zien dat co-stimulatie met R-spondin noodzakelijk is voor de optimale activatie van Wnt-signalering in een aantal myeloomcellijnen en in primaire myeloomcellen. Verlies van syndecan-1 HS zorgt dat het stimulerende effect van R-spondin op Wnt-signalering wegvalt. We tonen aan dat regulatie van Wnt-signalering door syndecan-1 mechanistisch gezien plaatsvindt door binding van Wnt-liganden en R-spondins aan de betreffende zijketens. Bovendien laten we zien dat het thrombospondin domein van R-spondin essentieel is voor het versterkende effect op Wnt-signalering en dat dit een belangrijke rol speelt bij de interactie tussen R-spondin en HS. Samengevat laat dit hoofdstuk zien dat de HS-zijketens die syndecan-1 bekleden in staat zijn Wnt en R-spondins te binden, resulterend in excessieve Wnt-signalering en versnelde celdeling in MM.

CXCL12 α wordt geproduceerd door stromale cellen in het beenmerg en speelt een belangrijke rol bij de homing en adhesie van myeloomcellen in de BM niche. De rol van CXCL12 γ , een product van alternatieve splicing, in de MM micro-omgeving is echter nog onbekend. In **hoofdstuk 4** laten we zien dat CXCL12 γ *in situ* tot expressie komt in zowel gezond beenmerg als in de MM beenmerg micro-omgeving en ook in uit het beenmerg geïsoleerde stromale cellen en stromale cellijnen. Belangrijk is dat, na uitscheiding, CXCL12 γ aanwezig blijft op het celoppervlak van deze stromale cellen door binding aan heparaan sulfaat proteoglycanen (HSPGs). Behandeling met recombinant CXCL12 γ zorgt voor sterke adhesie van myeloomcellen aan VCAM-1 en fibronectine. Interessant is dat deletie van zowel de CXCL12 γ isovorm als van EXT1 in BMSCs zorgt voor sterke vermindering van adhesie van myeloomcellen aan deze stromale cellen. Ook zorgt het voor verminderde bescherming tegen bortezomib-geïnduceerde celdood wat suggereert dat aan HSPGs gebonden CXCL12 γ een belangrijke rol speelt bij adhesie-gemedieerde drugresistentie. De data gepresenteerd in dit hoofdstuk impliceren dat CXCL12 γ functioneert als een zogenaamde 'niche chemokine', die tezamen met expressie van HSPGs, een unieke rol speelt bij het reguleren van MM adhesie, retentie in het beenmerg en drugresistentie.

In **hoofdstuk 5** behandelen we de cruciale rol van HSPGs in de interactie tussen normale en kwaadaardige plasmacellen en de beenmerg micro-omgeving. Na differentiatie van B-cellen tot plasma cellen wordt de B-cel-receptor (BCR) niet langer op de celmembranen tot expressie gebracht. De overleving van plasmacellen in het beenmerg is voornamelijk afhankelijk van overlevingssignalen afkomstig uit de BM micro-omgeving. Plasmacellen verwerven een hoge expressie van de HSPG syndecan-1. In deze review introduceren we eerst de B-celontwikkeling en de structuur, synthese en algemene functie van HSPG. Daarna bespreken we onderzoek dat laat zien dat syndecan-1 een essentiële rol speelt bij de ontwikkeling van B-cel tot plasmacel en bij de pathogenese van MM. Vervolgens beschrijven we hoe syndecan-1 de overleving van gezonde plasmacellen en myeloomcellen beïnvloedt.

Syndecan-1 stimuleert de overleving van gezonde plasmacellen en myeloomcellen door interactie met verschillende groeifactoren en cytokines zoals IL6, APRIL, HGF, Wnts, R-spondins en HB-EGF, die allemaal geproduceerd worden in de beenmerg micro-omgeving. Aansluitend bespreken we de HSPGs in de beenmergniche en focussen we met name op de rol van HSPGs bij adhesie en BMSC-geïnduceerde drugresistentie. Tenslotte bediscussiëren we klinische studies en huidige behandelingsmogelijkheden die aangrijpen op HSPGs en/of hun modificerende enzymen.

PhD portfolio

Name PhD student: Zemin Ren		
PhD period: 2012-2018		
Name PhD supervisor: Prof. Steven T. Pals		
1. PhD training		
	Year	Workload (Hours/ECTS)
Courses		
- Advanced immunology (UvA/VU/Sanquin)	2014	3
- Project management	2016	0.6
- Scientific Writing in English for Publication	2016	1.5
Seminars		
- Oncology seminar series (AMC)	2012-2017	4
- Immunology seminar series (AMC)	2012-2017	7.5
- Pathology seminar series (AMC)	2012-2017	4
- Weekly LYMMCARE meeting	2012-2018	9
Conferences		
- PhD retreat oncology (OOA), Renesse, NL	2013	1
- PhD retreat immunology (3I), Kameryck, NL	2013	1
- Pathology Research Day, Amsterdam, NL	2015	0.5
- Tumor cell biology conference (KWF), Lunteren, NL	2013-2017	5
- Molecular Aspects of Hematological Disorders, Rotterdam, NL	2016	1
- Dutch hematology conference (NVvH), Papendal, NL	2016,2018	2
- EMBO Wnt meeting, Brno, Czech Republic	2016	2
- Cancer Center Amsterdam retreat, Noordwijk, NL	2017	1

Presentations		
- Pathology Research Day, Amsterdam, NL	2015	0.5
- Molecular Aspects of Hematological Disorders, Rotterdam, NL	2016	0.5
- Tumor cell biology conference (KWF), Lunteren, NL	2016	0.5
- EMBO Wnt meeting, Brno, Czech Republic	2016	0.5
- Cancer Center Amsterdam retreat, Noordwijk, NL	2017	0.5
- Dutch hematology conference (NVvH), Papendal, NL	2018	0.5

2. Publications

Peer reviewed

- **Ren Z**, van Andel H, de Lau W, Hartholt RB, Maurice MM, Clevers H, Kersten MJ, Spaargaren M, Pals ST. Syndecan-1 promotes Wnt/beta-catenin signaling in multiple myeloma by presenting Wnts and R-spondins. **Blood**. 2018;131(9):982-994.
- van Andel H, **Ren Z**, Koopmans I, Joosten SPJ, Kocemba KA, de Lau W, Kersten MJ, de Bruin AM, Guikema JEJ, Clevers H, Spaargaren M, Pals ST. Aberrantly expressed LGR4 empowers Wnt signaling in multiple myeloma by hijacking osteoblast-derived R-spondins. **Proc Natl Acad Sci U S A**. 2017;114(2):376-381.
- Oud MM, Tuijnenburg P, Hempel M, van Vlies N, **Ren Z**, Ferdinandusse S, Jansen MH, Santer R, Johannsen J, Bacchelli C, Alders M, Li R, Davies R, Dupuis L, Cale CM, Wanders RJA, Pals ST, Ocala L, James C, Müller I, Lehmborg K, Strom T, Engels H, Williams HJ, Beales P, Roepman R, Dias P, Brunner HG, Cobben JM, Hall C, Hartley T, Le Quesne Stabej P, Mendoza-Londono R, Davies EG, de Sousa SB, Lessel D, Arts HH, Kuijpers TW. Mutations in EXTL3 Cause Neuro-immuno-skeletal Dysplasia Syndrome. **Am J Hum Genet**. 2017;100(2):281-296.

Manuscript in preparation

- **Ren Z**, Spaargaren M, Pals ST. Syndecan-1 and stromal HSPGs: key moderators of communication between normal and myeloma plasma cells and the bone marrow niche.
- **Ren Z**, Lantermans H, Kuil A, et al. CXCL12 γ immobilized by heparan sulfate proteoglycans on human bone marrow stromal cells instigates adhesion and drug resistance in multiple myeloma.

Curriculum Vitae

Zemin Ren was born on 26th of October 1985 in Datong, Shanxi, China. After he finished his high school study in 2003, he started to do coal business which his family was doing. One and half years later, he decided to go back to school for further education. In 2005, he started his undergraduate study in Veterinary Medicine in Shanxi Agriculture University. He was trained to become a veterinarian. In 2009, he received his bachelor degree. In 2011, he obtained the Certificate in Veterinary Practice.

In 2009, he started his master study in Immunology and Infectious Disease in Huazhong Agriculture University. From 2009 to 2012, he did research in Prof. Aizhen Guo's lab where he worked on the preparation of monoclonal antibodies and vaccines against *Mycoplasma bovis*, and one of monoclonal antibody he prepared resulted in a patent. In 2012, he received his master degree and he was awarded as Outstanding Graduate. In the same year, he won the Chinese National Scholarship which supported him to do Ph.D abroad.

In September 2012, he started his Ph.D study in the group of Prof. Steven T. Pals and Dr. Marcel Spaargaren in Amsterdam University Medical Center. He mainly focused on understanding the bone marrow microenvironment in multiple myeloma. His finding presented in this thesis identified HSPGs as key moderators of the interaction of multiple myeloma with the bone marrow niche.

Acknowledgments

I thought writing acknowledgments should be very easy, but now I realized that it is quite difficult, not because I don't have anything to say, but I have too much wanted to say. In 2012, with dream and curiosity, I came to the Netherlands to pursue my PhD. It was the first time I go abroad. Study in a completely different country is always not that easy, but it is also very exciting. During the last six years, I met so many nice people who supported me during this journey. I was born in a place where there is no word for "thanks" in our dialect. But it does not mean we do not know appreciation. Our appreciation is to remember the help in the deep of our heart and then hope to repay in the future. But now I would like to say thanks to people who helped me during this journey.

The first person I want to thank of course is my dear promoter prof. dr. Steven T. Pals. First I would like to thank you for accepting me as a PhD student in your group. Thank for your trust and your patience, especially in my first year when I was still in the early learning process. In the past few years, I have learned a lot from you, you always think in a clinical point of view which inspired me a lot. Thanks for your constructive suggestions and enthusiastic encouragement. In the last year of my PhD, you have spent a lot of time on my manuscripts and thesis. I know you are very busy with many clinical works. I appreciate it.

I would like also thank my co-promoter dr. Marcel Spaargaren. Marcel, it is you bring me to the Netherlands, which undoubtedly changed my entire life of course in a good way. That was a big step for me. Thanks for your trust. Your door was always open, many times I came to you and ask whether you have some time for discussion, your answer was always "yes", even though you were busy with your own work. I also appreciate that you helped me and went through my presentations and gave me a lot of good suggestions. Every time I learned something from you. After my Chinese scholarship is finished, you paid me which help me focus on the research. Thank you, Marcel.

I want to thank all the current and previous colleagues.

Harmen, you helped me to start my PhD life. I am grateful to you for teaching me important experimental skills and your help in my projects. During my PhD, when I had some difficulty with my project, I like to discuss it with you. This mostly happened in the late evening in the culture lab when we were still doing cell culture. I also enjoy those time we talk about football and basketball. Thank you for your help, constructive suggestion, and great company especially in the late evening and weekend in the lab.

Elena, I put your name here not because you are the girlfriend of Harmen. To me, you are like my big sister. You helped me a lot and you gave me a lot of sincere advice. I still remember, in my first week after I arrived in Netherland, I need to mail some documents to the Chinese embassy. You walk with me to the post office. And I still remember, you finished your experiment at 21:00 in the evening, you still take time to come to my office to teach

me how to quantify my western blot. Before you went to the US, I thought I might be gone when you come back. So I asked to take a photo of us. Thank you so much for your help and many suggestions for my research career and life. I hope to see you, Harmen, and Idris in China in the future.

Alex, although some of our projects did not work out. But you taught me a lot, flow cytometry is still one of my favorites technics. Even after you left, you still help me and gave me suggestions. Thank you, Alex! If I meet you next time, I hope you still remember the Chinese sentence you learned from me.

Martin, you are my Wikipedia. I have asked you a lot of questions including research, life in the Netherlands and even many nonsenses, you answered them all. Thank you, Martin. I enjoyed our birthday borrel very much, especially those before you went to NKI. After you come back from NKI, your party gene seems have a 50% knockdown. Maybe it's off target of your shRNA project there. I hope to meet you in China in the future, then I will treat you a lot of Chinese beer.

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Hildo, thanks for the help in the lab, many time when I need some cells, I can get from you. And thanks for switching to English when I am around, I really appreciated it. I still remember in the Nvvh meeting, you, me and Anna have to cross those iron fence gates to our hotel while other people were drinking indoor. It was a nice experience.

Jeroen, you once even saved my life. That time was the most dangerous time I have had. Fortunately, I have you to save me. Many times in the lab when I was not sure for something in my research, I went to your office to ask you. You always can give me good suggestions. I am very grateful for your suggestions and encouragement.

I also want to thanks many other colleagues in the B cell group, Marthe, big thanks for helping me with Dutch summary of my thesis. Timon, we started at the similar time. Thank you for your help and company in the past 6 years. It's great you started your postdoc position in the same group. Mahnoush, we were the only two foreigners in the B cell group and we also started our PhD in the same year. We support each other in the lab. I am glad you are still around in the AMC. I wish you get promoted soon and have a successful future. Guus, you always have funny jokes and a great sense of humor, it is a pleasure to have you as roommate. Nathalie, I wish we could have you in the group earlier, then I could learn dancing from you. I would like thanks other B cell group cool people Jerry, Yvonne, Ryan, Richard, Leonie, Willem, Annemieke, Anna, Maria for the help in the lab and fun company during my PhD study.

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任泽民

Zemin Ren

