Advanced microscopy studies of invadosome rosettes

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Publication date
2016
Document Version
Final published version

Addendum

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Advanced microscopy studies of invadosome rosettes

English summary

In this thesis we present studies that unravel the signaling and dynamic behavior of invadosomes, actin based structures that play an important role in cell migration. Moreover, we describe the development of new techniques with the potential to facilitate studies of both invadosomes and other dynamic biological processes.

Many cell types within a multicellular organism need to move around in order to perform their specific functions. For example, newly born neurons migrate from their birthplace to their final position in the brain, macrophages, which belong to the immune system, are constantly patrolling tissues in pursuit of invading pathogens, while osteoclasts travel towards bones before they can break down and remodel their surface. All these processes would be impossible without cellular migration. Unfortunately, the mechanism of migration is often hijacked by invasive cancer cells, which disseminate throughout the body in the process of metastasis.

In order to be able to move and migrate, cells first need to adhere to and then push through the extracellular matrix in which they are embedded. Interaction with the extracellular matrix is made possible by different types of cellular protrusions that are based on the internal musculoskeletal system of cells – the actin cytoskeleton. Among the different types of actin-based protrusions that are important for migrating cells, invadosomes are the ones that are the subject of this thesis. Invadosomes are unique in their ability not only to exert force, but also to secrete enzymes degrading extracellular matrix. This degradation step is necessary in the process of migration when cells are neither able to squeeze through nor to mechanically remodel tightly woven regions of extracellular matrix. Only certain cell types are capable of producing invadosomes and degrading extracellular matrix, these are for example patrolling cells of our immune system or metastasizing cancer cells.

Invadosomes can be rapidly remodeled following chemical and mechanical cues coming from the extracellular environment. It is possible due to the versatile group of proteins that are constantly building and rearranging actin networks within invadosomes. As our knowledge about invadosomes, their function and remodeling has expanded in recent years, in **Chapter 2** we review the current understanding of these structures. In this review we specifically focus on the internal actin-based architecture of invadosomes and their capacity to adjust to mechanical properties of the extracellular matrix.

Interestingly, invadosomes often do not function alone. They can arrange themselves into higher order structures like static clusters, dynamic circular rosettes or

belts at the periphery of cells. Acting together, they can exercise functions that they cannot on their own. For example, they can effectively follow topography of a substrate and increase their degradative properties. So what are the signaling steps controlling arrangement of invadosomes into their higher order structures? We addressed this question in a study presented in **Chapter 3**.

In order to understand signaling events behind the rearrangement of invadosome clusters into dynamic invadosome rosettes we used melanoma cells transformed with a potent oncogene (Src), as these cells spontaneously produced prominent clusters of invadosomes at their periphery. We observed that when these cells were stimulated with certain agonists that bind a specific class of receptors (G protein coupled receptors), the stable clusters were rapidly remodeled into fast moving invadosome rosettes. We focused our studies on one of the rosettes-inducing agonists, lysophosphatidic acid (LPA) because it is a signaling lipid that plays a prominent role in migration and dissemination of many kinds of cancer cells, including melanoma cells. We found that LPA stimulates development of rosettes acting specifically through the previously characterized LPA1 – Gα_i – PI3K signaling pathway. This pathway activates Cdc42, a small protein that can promote rearrangement of actin networks to form invadosomes. Interestingly, LPA simultaneously stimulated RhoA, a protein which showed an inhibitory effect towards rosettes. Therefore, our results revealed that the development of rosettes in Src-transformed melanoma cells is controlled by a fine balance between stimulatory and inhibitory signals.

We also found that LPA-induced rosettes are extremely dynamic. In order to study and understand the principles of their dynamic behavior we developed and characterized a tool that allows us to stimulate cells in a precisely controlled way: caged LPA (**Chapter 4**). Caged LPA is a chemically modified form of LPA that becomes biologically active only when illuminated with a specific wavelength (color) of light. As light of a defined wavelength can be easily delivered to a selected spot in a microscopy experiment, cgLPA allowed us to stimulate cells with unmatched precision.

Using caged LPA we studied the dynamic behavior of invadosome rosettes in Src-transformed melanoma cells (**Chapter 5**). For example we observed that invadosome rosettes developed only at spots that were directly stimulated by LPA, and that cells migrating towards a source of LPA preferentially produced rosettes at their leading edges. These observations suggest a specific role of rosettes in LPA-induced migration. Most importantly we observed that rosettes in their expansion and directional movement were guided by a thin veil of actin – the ventral lamellipodium. This kind of cooperation between invadosome rosettes and ventral lamellipodia is an interesting example of how different actin-based structures team up to enable cells to migrate.

Finally, in **Chapter 6** we described the development of a new technique, siFLIM that will be useful to study fast and dynamic biological processes like the expansion of invadosome rosettes. siFLIM (Single Image Fluorescence Lifetime Imaging Microscopy) makes it possible to acquire lifetime data with a very high speed. FLIM enables measurement of the physicochemical properties of the cellular environment, for example pH or can even provide information about interactions between different proteins. FLIM measurements can be challenging when conditions change quickly in dynamic biological processes. We demonstrate that in these demanding conditions siFLIM provides reliable lifetime data.

Our studies elucidate selected aspects of signaling and dynamic behavior of invadosomes. Hopefully, this will contribute towards controlling cell migration in disease, for example in cancer. Furthermore, we envision that the new tools presented in this thesis, caged LPA and siFLIM will be useful for future studies in the field of invadosomes and beyond.

Invadosoomrosetten bestudeerd met geadvanceerde misroscopie

Nederlandse samenvatting

In dit proefschrift beschrijven we studies die de signaaltransductie en het dynamische gedrag ontrafelen van invadosomen, op actine gebaseerde structuren die een belangrijke rol spelen bij cel migratie. Daarnaast beschrijven we de ontwikkeling van nieuwe technieken die nuttig kunnen zijn bij het bestuderen van invadosomen en andere dynamische biologische processen.

Veel celtypen in multi-cellulaire organismen moeten zich verplaatsen om hun specifieke functies uit te oefenen. Pasgevormde neuronen, bijvoorbeeld, migreren van hun geboorteplaats naar hun vaste plek in het brein, macrofagen – cellen van het immuunsysteem – patrouilleren constant in weefsels op jacht naar binnendringende pathogenen, terwijl osteoclasten afreizen naar botten om hun oppervlak af te breken en te herstructureren. Al deze processen zouden onmogelijk zijn zonder cellulaire migratie. Helaas wordt het mechanisme van migratie vaak gekaapt door invasieve kankercellen, die zich in het lichaam verspreiden in een proces dat metastase heet.

Om te kunnen migreren moeten cellen allereerst hechten aan de extracellulaire matrix (ECM) waarin ze zijn ingebed, en zich hier vervolgens een weg door banen. De interactie met de extracellulaire matrix geschiedt door middel van verscheidene typen uitstulpingen van de cel, die berusten op het musculoskeletale systeem van de cel: het actine cytoskelet. Invadosomen, het onderwerp van dit proefschrift, vormen één zo'n type uitstulping. Uniek voor invadosomen is dat ze niet alleen kracht uitoefenen op de extracellulaire matrix, maar ook enzymen uitscheiden die de matrix afbreken. Deze degradatiestap is vaak nodig bij migratie, als cellen ECM tegenkomen die zo dicht is dat ze er niet doorheen kunnen kruipen of opzij kunnen duwen. Niet alle celtypen kunnen invadosomen maken en de extracellulaire matrix afbreken; dat is beperkt tot celtypen zoals de patrouillerende immuuncellen of metastaserende kankercellen.

Een uiteenlopende groep van eiwitten verzorgen de opbouw en structuur van de actinenetwerken in invadosomen. Deze zorgen ook dat invadosomen snel kunnen reageren op ECM signalen van chemische of mechanische aard. De afgelopen jaren is ons begrip van structuur, functie en dynamica van invadosomen flink toegenomen. Daarom geven we in **Hoofdstuk 2** een overzicht van de huidige staat van kennis van deze structuren. In deze review focussen we specifiek op de interne actinestructuur van invadosomen en hun vermogen om de mechanische

eigenschappen van de extracellulaire matrix aan te passen.

Invadosomen werken meestal niet alleen. Ze kunnen hogere-orde structuren vormen, zoals stabiele clusters, dynamische ronde rozetten of gordels aan de buitenkant van de cel. Zulke hogere-orde structuren kunnen functies uitoefenen waarover losse invadosomen niet beschikken. Ze kunnen bijvoorbeeld doeltreffend de topologie van een substraat volgen en de ECM veel efficiënter afbreken. Welke signaleringstappen coördineren de ordening van invadosomen in hogere orde structuren? Deze vraag hebben we bestudeerd in **Hoofdstuk 3**.

Om te begrijpen welke signaaltransductiestappen de transformatie van statische invadosoomclusters naar dynamische invadosoomrozetten teweeg brengen hebben we melanoomcellen getransformeerd met een krachtig oncogen (Src), omdat de cellen dan spontaan markante invadosoomclusters aan hun buitenrand produceren. We hebben gezien dat na stimulatie met een agonist die bindt aan een specifieke receptorklasse (G-proteïne gekoppelde receptoren) de stabiele clusters snel werden omgevormd tot heel bewegelijke invadosoomrozetten. Hierbij hebben we ons voornamelijk gericht op één van de rozet-producerende agonisten, lysofosfatidinezuur (LPA), omdat deze signaaltransductielipide een prominente rol speelt bij migratie en disseminatie van vele soorten kankercellen, waaronder melanoomcellen. We hebben gevonden dat LPA specifiek rozetformatie stimuleert via het eerder gekarakteriseerde LPA1– Gα_i – PI3K signaaltransductiepad. Hierbij wordt Cdc42 geactiveerd, een klein eiwit dat herstructurering van actinenetwerken bevordert. Opmerkelijk genoeg stimuleert LPA gelijktijdig RhoA, een eiwit dat juist een remmend effect op rozetten heeft. Onze resultaten onthullen dus dat de ontwikkeling van rozetten in Src-getransformeerde melanoomcellen beheerst wordt door een subtiele balans tussen bevorderende en belemmerende signalen.

We hebben ook gezien dat LPA-geïnduceerde rozetten extreem dynamisch zijn. Om de principes van hun dynamische gedrag te doorgronden hebben we een manier ontwikkeld en gekarakteriseerd waarmee cellen op gecontroleerde wijze kunnen worden gestimuleerd: 'caged LPA' (cgLPA) (**Hoofdstuk 4**). cgLPA is een chemisch gemodificeerde vorm van LPA dat alleen biologisch actief wordt wanneer het wordt blootgesteld aan UV licht. cgLPA stelt ons in staat cellen te stimuleren met ongeëvenaarde precisie. We hebben dit gebruikt om het gedrag van invadosoomrozetten in Src-getransformeerde cellen in detail te bestuderen (**Hoofdstuk 5**). Uit deze studies bleek dat rozetten alleen ontstaan op de plekken die rechtstreeks gestimuleerd werden met LPA, en dat cellen die in een gradiënt van LPA migreren bij voorkeur rozetten produceren aan hun *leading edges*. Deze observaties suggereren dat rozetten een rol spelen in LPA-geïnduceerde migratie. Noemenswaardig is dat de groeiende rozetten begeleid werden door een dunne sluier van actine – het ventrale lamellipodium. Deze samenwerking tussen invadosoomrozetten en ventrale lamellipodia is een interessant voorbeeld van hoe verschillende actinestructuren

hun krachten bundelen tijdens celmigratie.

Ten slotte beschrijven we in **Hoofdstuk 6** de ontwikkeling van een nieuwe techniek, siFLIM, die van nut zal zijn om snelle en dynamische biologische processen te bestuderen, zoals expanderende invadosoomrozetten. siFLIM (*single image Fluorescence Lifetime Imaging Microscopy*) maakt het mogelijk om de fluorescentie levensduur van indicatoren snel en efficiënt te meten. Met FLIM kunnen fysisch-chemische eigenschappen van de cellulaire omgeving worden bepaald (zoals pH, zuurstofconcentratie etc.) en interacties tussen eiwitten in kaart gebracht. In het verleden was het lastig om bij dynamische biologische processen dit soort data te meten. We demonstreren hier dat siFLIM ook in deze veeleisende condities betrouwbare levensduurdata oplevert.

Ons werk belicht diverse aspecten van de signaaltransductie en het dynamisch gedrag van invadosomen. De resultaten leveren een bijdrage aan het begrip – en hopelijk uiteindelijk ook de beheersing – van celmigratie bij ziekten, bijvoorbeeld bij kanker. Bovendien verwachten we dat de nieuwe gereedschappen die gepresenteerd worden in dit proefschrift, caged LPA en siFLIM, gebruikt gaan worden in toekomstige studies in het invadosoomveld en daarbuiten.

Curriculum Vitae

Katarzyna (Kasia) Kedziora was born on December 10th, 1984 in Zawiercie, Poland. She obtained her high school diploma from the I Liceum Ogolnoksztatcace in Zawiercie in 2003. That same year she enrolled in the MSc program in Biophysics at the Jagiellonian University in Cracow, Poland. During her Master internship in the Cell Biophysics group of prof. Jerzy Dobrucki she studied dynamics of PCNA protein and its role in DNA repair processes. She graduated and obtained MSc degree in 2008. In 2006 she joined the Bachelor of Engineering program in the School of Engineering in Biomedicine at the AGH University of Science and Technology in Cracow. She performed her internship in the Laboratory of Cryptography and Cognitive Informatics under the supervision of prof. Marek Ogiela where she studied the potential of machine learning algorithms in the classification of cell cycle phases based on confocal images of chromatin. She obtained her B.Eng degree in 2010. That same year she followed a research training in the Van Leeuwenhoek Center for Advanced Microscopy at the University of Amsterdam. During this internship she was part of a team that developed a user interface to control a prototype WF-CLEM microscope. In October 2010 she began her PhD training in the Biophysics group of prof. Kees Jalink in the Netherlands Cancer Institute (NKI). Her project, funded by the Dutch Cancer Society, was focused on studying signaling and dynamics of invadosomes and the results of this research are described in this thesis. She will continue her scientific career as a postdoctoral fellow in the group of prof. Jeremy Purvis in the Department of Genetics at the University of North Carolina at Chapel Hill.

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List of publications

Within this thesis

Kedziora KM, Leyton-Puig D, Innocenti M, Jalink K. Dynamic rosettes of invadosomes are driven by ventral lamellipodia.
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Acknowledgements

Most of the important things we do, we do with others. Some students think that research is different: they imagine a solitary scholar reading alone in a hushed library or peering into a microscope surrounded only by glassware and computers. But no place is more filled with voices than a library or lab.

W. C. Booth, G. G. Colomb, J. M. Williams: The Craft of Research

Above words beautifully capture the collaborative essence of science. As many scientific collaborations also shaped the above thesis I wish to express my deepest gratitude to everybody who was involved in this work. I thank all of you for anything that is valuable in this thesis while I take the full responsibility for everything that should be better.

Kees, we always shared the love of science but you showed me how to control this love without losing you. You taught me how to question all assumptions, how to follow my intuition without really believing it and how to come back to the drawing board when a control of a control didn't work. I'm grateful for all your lessons, long discussions in front of a microscope and all the freedom of making my own mistakes. In all my future experiments there will be a part of you and it will be the best part of them.

Wouter, thank you for teaching me that time is limited and that enthusiasm alone is not enough if it is not focused on the most important question. The pursuit of such a question and its answer often means choosing the most difficult pathway so thank you for making me ready to make this choice.

Metello, I admire your attitude towards science, your critical approach and your persistence. Thank you for allowing me to be a part of a fascinating mDia story and for always being there for me. You are an infinite source of ideas and they are the best illustration for me of the infinite potential of research.

I was fortunate to work with a group of people who shared with me scientific projects and enthusiasm towards life. **Daniela**, thank you for all the amazing discussions about both proteins and people. I admire your balanced approach towards experiments and life and your ability to forsake this balance in order to pursue what is really worth it. We shared many weekends in the lab, cups of coffee and blinking flurophores. I want you to know that I wouldn't make it without you. **Bram**, it was so great to exchange all the ideas with you. Your calm attitude and your belief that there is a solution to any problem taught me

to take a deep breath and recalculate everything once again... Thank you for always finding time to answer my countless questions. I still have no idea how you managed to do that. **Tada**, thank you for all your kindness and hard working attitude. I believe that science is the way to make world a better place and I'm so sure that you will find a way to do it. I'm deeply grateful for all the experiments that we shared and words that we wrote together. **Jeffrey**, thank you for taking such a great care of the lab, your help in experiments, brainstorming about FRET and never getting angry when your black markers naturally gravitate towards my lab bench. **Daan**, thank you for being my 'older PhD brother'. You generously shared with me not only your favorite protein, pipetting tricks and the love towards podosomes but also so many discussions about life... and Kees. Thank you for letting me take care of Intercity Young Scientists Meeting. I learned so much organizing it. **Marcel**, thank you for our countless discussions about siFLIM, your patience, your practical approach to problems which I were repeatedly over-complicating and testing the capacity limits of PowerPoint for us. **Leila**, thank you for your enthusiasm towards microscopy, all fascinating conversations during lunch and Persian poems. **Pablo**, thank you for your warmth, discussing books next to our coffee machine and putting LPA and integrins in one sentence. **Elisa**, your inexhaustible energy, precision in doing experiments and smile were such a great part of our lab atmosphere. Thank you for always being there to help, for your positive attitude, for sharing all the fascinating details about GDE2 and for Spanish lessons. **Elisabetta**, thank you for sharing with me translocations, FRET experiments, fluorescent lipids and phospho-blots - we explored together so many different projects! I admire your perfection in doing experiments and I'm grateful for your patience with me putting a membrane in wrong positions... **Michiel**, it is so great that you always look for better approaches and techniques. Thank you for sharing with me your most amazing GDE3 protein. **Maaike**, I so loved your smile, great appreciation of amazing Dutch weather and ability to clone any of Arnoud's ideas. Thank you for saving my life with coffee supplies, sharing love towards chocolate and being patient with my attempts to speak Dutch. Many thanks to all the people who made B5 such a good place to do science - members of Sonnenberg group: **Arnoud**, who loves integrins as much as chocolate; **Norman**, thank you for teaching me so many things about the NKI; **Coert**, thank you teaching me so much about micropatterns and nucleus shape; **Veronika**, we shared so many evenings in the lab together; **Lisa** and **Alba**, unstoppable pursuers of adhesive molecules; all the members of Wolthuis, Medema and Rowland groups. Many thanks also to our great collaborators in Nijmegen - **Frank**, **Jeroen** and **Arthur**, it was always great to discuss invadosomes with you.

Anja and **Bram**, my great students, you taught me so much more than I was able to teach you. Thank you for your enthusiasm towards rosettes, for constantly

being open to try new experiments and for your patience with my far-fetched ideas.

Lauran and **Lenny** thank you for all the technical help with sometimes misbehaving microscopes. **Rob vd K.**, thank you for your great technical help in experiments; **Minze** and **Erwin**, thank you for taking care of my frozen cells. **Rob K.**, thank you for taking care of the lab and solving all the issues so smoothly; **Patty**, **Marianne** and **Mariet** thank you for all your help with administrative things.

My friends, **Sjuul** and **Erik**, thank you for your enormous help and hospitality. You shared with me so many moments, both good and bad. Although, we would all wish only for the first category, these is the second one that defines a true friendship. **Christine**, thank you for discussions about science, cups of good tea, yoga classes and our pursuit for more balance in our lives.

Drodzy **Rodzice**, dziękuję, że zawsze pozwalaliście mi wybrać w życiu to, co było dla mnie najbardziej fascynujące i wspieraliście mnie w każdym z tych wyborów. Dziękuję za Wasz uśmiech, tysiące pytań o to czym się zajmuję i sprawdzanie każdego dnia czy jestem szczęśliwa.

Michał, ta rozprawa nigdy nie zostałaby napisana, gdybyś we mnie nie wierzył, dlatego dedykuję ją Tobie. Każdego dnia jesteś dla mnie źródłem motywacji i siły, dziękuję Ci więc za każdy dzień.

Дмитрий, спасибо за за твою повседневную поддержку. Благодарю тебя за твой интерес к многим вещам, включая розетты. Ты помог в написании этой диссертации многими различными способами. Ты наблюдал её развитие день ото дня. Ты всегда был рядом, чтобы напомнить мне, что это не самое главное в жизни. спасибо.