

Mechanisms of Ewing sarcoma metastasis : biochemistry and biophysics Beletkaia, E.

Citation

Beletkaia, E. (2015, December 9). *Mechanisms of Ewing sarcoma metastasis : biochemistry and biophysics*. Retrieved from https://hdl.handle.net/1887/37000

Version:Not Applicable (or Unknown)License:Leiden University Non-exclusive licenseDownloaded from:https://hdl.handle.net/1887/37000

Note: To cite this publication please use the final published version (if applicable).

Cover Page



Universiteit Leiden



The handle <u>http://hdl.handle.net/1887/37000</u> holds various files of this Leiden University dissertation.

Author: Beletkaia, Elena Title: Mechanisms of Ewing sarcoma metastasis : biochemistry and biophysics Issue Date: 2015-12-09

Mechanisms of Ewing Sarcoma Metastasis

Biochemistry and Biophysics

PROEFSCHRIFT

ter verkrijging van de graad van Doctor aan de Universiteit Leiden, op gezag van Rector Magnificus prof. mr. C.J.J.M. Stolker, volgens besluit van het College voor Promoties te verdedigen op woensdag 9 december 2015 klokke 10.00 uur

 door

Elena Beleţkaia

geboren te Chişinău (Moldova) in 1989

Promotor:	Prof. dr. T. Schmidt
Co-promotor:	Dr. B. E. Snaar-Jagalska

Promotiecommissie:

Dr. S. F. Fenz (Julius-Maximilians University, Würzburg, Germany)
Prof. dr. M. Bünemann (Philipps University, Marburg, Germany)
Prof. dr. M. Dogterom
Prof. dr. E. Eliel
Prof. dr. P. C. W. Hogendorn

ISBN 978-90-8593-234-5 Casimir PhD series Delft-Leiden 2015-28 An electronic version of this thesis can be found at https://openaccess.leidenuniv.nl

This work is part of the research programme TOPGO.L.10.064, which is financed by the Netherlands Organisation for Scientific Research (NWO).

For Katherine

CONTENTS

1	Intr	roduction 1				
	1.1	Ewing sarcoma				
	1.2	Ewing sarcoma's origin				
	1.3	EWS/FLI fusion protein				
	1.4	CXCR4 6				
		1.4.1 CXCR4 structure				
		1.4.2 Activation mechanism				
		1.4.3 CXCR4 signaling				
		1.4.4 CXCR4 in cancer metastasis				
	1.5	Ewing sarcoma metastasis				
		1.5.1 CXCR4 in ES metastasis $\ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots 10$				
		1.5.2 Other modulators of ES metastasis 10				
		1.5.3 Anchorage-independent growth				
	1.6	Cancer mechanics				
		1.6.1 Cellular stiffness $\ldots \ldots 12$				
		1.6.2 Role of the extracellular matrix				
	1.7	Thesis outline				
	1.8	Appendix				
	1.9	References				
2	CX	CR4 signaling control 29				
	2.1	Introduction				
	2.2	Materials and Methods				
		2.2.1 Cell culture and transfection				
		2.2.2 Sample preparation				
		2.2.3 Global CXCL12 stimulation assay				
		2.2.4 Calcium assay				
		2.2.5 Actin depolymerization				

		2.2.6	Endocytosis inhibition	34
		2.2.7	$G_{\alpha i}$ inhibition	34
		2.2.8	Single-molecule imaging	34
		2.2.9	Time-lapse microscopy	36
		2.2.10	Simulation of diffusion on a vesicle	36
	2.3	Result	8	37
		2.3.1	CXCR4-eYFP is functional in A673-CXCR4 cells .	37
		2.3.2	CXCR4 do not homodimerize upon activation in	
			A673 cells	39
		2.3.3	Activation of CXCR4 causes immobilization of the	
			receptors	41
		2.3.4	Actin cytoskeleton is not responsible for CXCR4	
			immobilization	43
		2.3.5	CXCR4 in endocytotic vesicles contribute to the	
			immobile receptor fraction	45
		2.3.6	G-proteins control CXCR4 immobilization	47
		2.3.7	Cross-talk between G-protein dependent and inde-	
			pendent pathways	48
	2.4	Discus	sion	50
	2.5	Supple	emental figures	55
	2.6	Ackno	wlegement	57
	2.7	Refere	nces	58
3	G-p	rotein	activation mode by CXCR4	65
	3.1	Introd	uction	67
	3.2	Materi	als and Methods	69
		3.2.1	Cell culture and transfection $\ldots \ldots \ldots \ldots$	69
		3.2.2	Confocal microscopy	69
		3.2.3	Single-molecule imaging	69
	3.3	Result	\mathbf{sults}	
		3.3.1	Receptor interaction is required for G-protein lo-	
			calization to the plasma membrane \hdots	70
		3.3.2	Mobility of membrane proteins is unaltered on cell-	
			substrate adhesion $\ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots$	74
		3.3.3	Binding of $G_{\alpha i}$ to CXCR4 requires receptor acti-	
			vation	76
		3.3.4	$G_{\alpha q}$ is pre-coupled to an inactive CXCR4 receptor	78
	3.4	Discus	sion \ldots	79
	25	Aclence	wlogomont	82

	3.6	References	33
4	Cor	nstruction of optoCXCR4 8	37
	4.1	Introduction	39
	4.2	Materials and Methods	91
		4.2.1 Cell culture	91
		4.2.2 Sequences alignments	91
		4.2.3 Whole-plasmid cloning	92
		4.2.4 Cell transfection	92
		4.2.5 Microscopy	92
		4.2.6 Dual reporter activity assay	93
	4.3	Design and characterization of an optoCXCR4 receptor 9	94
		4.3.1 Design of optoCXCR4	94
		4.3.2 Cloning of various optoCXCR4 constructs 9	96
		4.3.3 Optimization of cell transfection	99
		4.3.4 Localization of the chimeric receptors)0
		4.3.5 Functionality of the optoCXCR4 constructs 10)2
		4.3.6 Fast optoCXCR4 internalization)3
	4.4	Conclusions )4
	4.5	Supplemental figures)6
	4.6	Acknowlegement)8
	4.7	References)9
5	Me	chanics of ES metastasis 11	13
	5.1	Introduction	15
	5.2	Materials and Methods	16
		5.2.1 Cell culture and viral transfection	16
		5.2.2 Collagen gel assay	17
		5.2.3 Micropillar preparation	17
		5.2.4 Microscopy	18
		5.2.5 Force detection $\ldots \ldots 11$	19
		$5.2.6$ Immunostaining $\ldots \ldots 11$	19
		5.2.7 Cell area detection	19
		5.2.8 2D migration rate detection	19
	5.3	Results	20
		5.3.1 Cell phenotype depends on substrate modification 12	20
		5.3.2 Cell invasion in 3D is stiffness dependent 12	22
		5.3.3 Ewing sarcoma cells change their mechanical phe-	
		notype on micropillar arrays of varying stiffness . 12	24

	5.3.4	Dynamic force development depends on substrate stiffness	126
	5.3.5	Activation of cells did not alter their morphological	
		and mechanical phenotype	128
5.4	Discus	ssion \ldots	130
5.5	Supple	emental figures	133
5.6	Refere	ences	136
Summ	ary	-	141
Samen	vatting	g	145
Public	ations	3	149
Curric	ulum V	Vitae	151