

Table of contents

Introduction	1
1.1. Breast cancer.....	3
1.1.1. Epidemiology.....	3
1.1.2. Risk factors	4
1.1.3. Screening and diagnosis	6
1.1.4. Classification and staging.....	7
1.1.4.1. Histological and anatomical classification	7
1.1.4.2. Pathological classification.....	10
1.1.4.3. Molecular classification.....	11
1.1.5. Treatment.....	13
1.2. Tumor microenvironment	17
1.2.1. Cancer-associated fibroblasts	21
1.2.1.1. Origin and heterogeneity	21
1.2.1.2. Implications in tumor microenvironment.....	26
1.2.1.3. Implications in tumor progression and therapy resistance	29
1.3. Nanoparticles in medicine	36
1.3.1. Mesoporous silica nanoparticles.....	41
1.3.2. Modulation and targeting of tumor microenvironment by nanoparticles.....	45
1.3.2.1. Nanoparticles targeting cancer-associated fibroblasts ...	
.....	54

TABLE OF CONTENTS

Objectives.....	57
Materials and Methods	61
3.1. Cell culture	63
3.1.1. Commercial cell lines	63
3.1.2. Cancer-associated fibroblasts.....	63
3.1.3. Patient-derived organoids	65
3.2. Gene expression studies	67
3.3. Western blot	68
3.4. Nanoparticles' synthesis	69
3.5. Nanoparticles' characterization	70
3.6. Control release studies	71
3.7. Hemolysis assay.....	72
3.8. Cellular uptake and targeting efficacy	73
3.8.1. 2D culture models.....	73
3.8.2. 3D culture models.....	73
3.9. Cellular cytotoxicity.....	74
3.9.1. 2D culture models.....	74
3.9.2. 3D culture models.....	75
3.10. <i>In vivo</i> studies.....	76
3.10.1. <i>In vivo</i> model.....	76
3.10.2. Doxorubicin penetration	77
3.10.3. Masson's trichrome staining.....	77
3.10.4. Immunofluorescence.....	78

TABLE OF CONTENTS

3.10.5. Flow cytometry	78
3.11. Statistical analysis.....	80
Results	81
4.1. Synthesis and characterization of nanoparticles targeting cancer-associated fibroblasts.....	83
4.2. FAP- α expression in breast cancer cells, cancer-associated fibroblasts, and patient-derived organoids.....	91
4.3. Nanoparticles' targeting efficacy in breast cancer cells, cancer-associated fibroblasts, and patient-derived organoids	94
4.4. Nanoparticles' cytotoxic efficacy in breast cancer cells, cancer-associated fibroblasts, and patient-derived organoids	100
4.5. Nanoparticles' <i>in vivo</i> antitumoral efficacy in a triple-negative breast cancer mouse model.....	102
4.6. Nanoparticles' <i>in vivo</i> targeting of cancer-associated fibroblasts	106
4.7. Nanoparticles' <i>in vivo</i> tumor microenvironment modulation .	107
4.8. Nanoparticles' <i>in vivo</i> biocompatibility and safety	110
Discussion	115
Conclusions.....	123
Bibliography	127
Appendix.....	175