MULTIFUNCTIONAL NANOMEDICINES BASED ON ALBUMIN FOR TARGETED BREAST CANCER THERAPY

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Declarations

Statement of Originality

I hereby certify that to the best of my knowledge and belief this thesis is my own work and contains no material previously published or written by another person except where due references and acknowledgements are made. It contains no material which has been previously submitted by me/others for the award of any other degree or diploma in any university or other tertiary institution. I give consent to the final version of my thesis being made available worldwide when deposited in the University's Digital Repository[**], subject to the provisions of the Copyright Act 1968.
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I hereby certify that the work embodied in this thesis contains published papers/scholarly works of which I am a joint author. I have included as part of the thesis a written statement, endorsed by my supervisor, attesting to my contribution to the joint publications/scholarly works.

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I hereby certify that this thesis is in the form of a series of papers of which I am a joint author. I have included as part of the thesis a written statement from each co-author, endorsed by the Faculty Assistant Dean (Research Training), attesting to contribution to the joint publications.

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ABSTRACT

Albumin can promote intratumoural drug accumulation and uptake due to its interaction with albumin-binding proteins in tumour microenvironment, therefore albumin has emerged as an attractive drug carrier for cancer diagnosis and treatment. Tropomyosin receptor kinase A (TrkA) is regarded as one of the potential therapeutic targets in oncology and, in breast cancer, is associated with the stimulation of tumour growth and metastasis. By taking advantage of these two perspectives, in this project, we have developed novel albumin-based nanoparticles (NPs) to enable targeted delivery of TrkA inhibitors for breast cancer therapy.

In the first part of this project, novel albumin hybrid NPs (Alb-HNPs) loaded with a selective TrkA inhibitor GNF-5837 were prepared and evaluated for antineoplastic efficacy in a panel of breast cancer cell lines. The nanomedicines (GNF-Alb-HNPs) were formed through a unique polyelectrolyte complexation process where albumin and GNF-5837 were encapsulated by a stabilising layer of oppositely charged chitosan and dextran sulphate polysaccharides. GNF-Alb-HNPs showed a particle size of ~150 nm and excellent colloidal stability, which makes them ideal for passive targeting to tumours through the enhanced permeability and retention (EPR) effect. GNF-Alb-HNPs were shown to specifically inhibit TrkA phosphorylation and downstream mitogen-activated protein kinase (MAPK) signalling in MDA-MB-231 breast cancer cells, resulting in anti-proliferative and pro-apoptotic effects. Compared with the free GNF-5837, the GNF-Alb-HNPs not only exhibited an enhanced anti-proliferative and anti-invasive effects but also significantly increased the apoptosis of cancer cells.

In the second part of this project, we aimed to develop on-demand Alb-HNPs that can respond to the tumour microenvironment and facilitate deep tumour penetration. Gelatine-albumin hybrid NPs (Gel-Alb HNPs) were developed for the delivery of the TrkA inhibitor GNF-5837, where the overexpression of matrix metalloproteinase (MMP) enzymes in the tumour can trigger site-specific release of the small drug-bound albumins for efficient uptake by cancer cells. The nanomedicines (Gel-Alb-GNF HNPs) were prepared using a pH-controlled complexation process from the pre-synthesised cationic gelatine, dextran sulphate and albumin-bound GNF-5837. Gel-Alb-GNF HNPs had a particle size of ~130 nm with narrow size distribution (polydispersity index: 0.15). They displayed excellent colloidal stability but disassembled in the presence of MMP-2 which is elevated in the extracellular matrix of tumours and can degrade

cationic gelatine. Gel-Alb-GNF HNPs were shown to significantly inhibit malignant TrkA phosphorylation and downstream MAPK or Akt signalling in breast cancer cells but markedly increased their caspase-dependant apoptosis. Moreover, the migration and invasion activities of cancer cells were dramatically suppressed and the inhibitory effects were more prominent with Gel-Alb-GNF HNPS than the GNF-Alb-HNPs.

Overall, results suggest that both Alb-HNPs and Gel-Alb HNPs are able to markedly improve the efficacy and specificity of encapsulated TrkA inhibitor GNF-5837 for breast cancer therapy. As TrkA receptor has been implicated in chemosensitisation as well as neuropathic pain, it is anticipated that these novel therapeutic approaches will be adaptable for the treatment of chemotherapy resistance and cancer associated pain.

List of publications included as part of the thesis

CONTAINED IN:

CHAPTER 2

Shargh, V.H., Hondermarck, H., Liang, M., 2016. **Albumin Hybrid Nanoparticles Loaded with Tyrosine Kinase A Inhibitor GNF-5837 for Targeted Inhibition of Breast Cancer Cell Growth and Invasion.** *International Journal of Pharmaceutics* 515, 527-534.

CHAPTER 3

Shargh, V.H., Hondermarck, H., Liang, M. **Gelatine–albumin hybrid nanoparticles as matrix metalloproteinases-degradable delivery systems for breast cancer therapy.** In Press: *Nanomedicine (Lond).*

CHAPTER 5

Shargh, V.H., Hondermarck, H., Liang, M., 2016. **Antibody-targeted biodegradable nanoparticles for cancer therapy.** *Nanomedicine (Lond)* 11, 63-79.

Statement from co-authors relating to papers published with Vahid Heravi Shargh

I, the undersigned corresponding author of the following papers:

- Shargh, V.H., Hondermarck, H., Liang, M., 2016. **Albumin Hybrid Nanoparticles Loaded with Tyrosine Kinase A Inhibitor GNF-5837 for Targeted Inhibition of Breast Cancer Cell Growth and Invasion.** *International Journal of Pharmaceutics* 515, 527-534.
- Shargh, V.H., Hondermarck, H., Liang, M. **Gelatine–albumin hybrid nanoparticles as matrix metalloproteinases-degradable delivery systems for breast cancer therapy.** In Press: *Nanomedicine (Lond).*
- Shargh, V.H., Hondermarck, H., Liang, M., 2016. **Antibody-targeted biodegradable nanoparticles for cancer therapy.** *Nanomedicine (Lond)* 11, 63-79.

Authorize the inclusion of these works and declare that Research Higher Degree candidate Vahid Heravi Shargh contributed to the fulfillment of the papers as outlined below:

- To the design and conduct of most experiments
- To the critical analysis and interpretation of the results
- To the preparation and organisation of the figures and tables
- To the drafting and conceptualisation of the manuscripts
- To the revision of the manuscripts

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I, the undersigned co-author of the following papers:

- Shargh, V.H., Hondermarck, H., Liang, M., 2016. **Albumin Hybrid Nanoparticles Loaded with Tyrosine Kinase A Inhibitor GNF-5837 for Targeted Inhibition of Breast Cancer Cell Growth and Invasion.** *International Journal of Pharmaceutics* 515, 527-534.
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Conference Presentations

- Heravi Shargh V, Hondermarck H, Liang, M. (2016) Enhanced anticancer activity of tyrosine kinase inhibitor GNF-5837 via tumour responsive gelatine-albumin hybrid nanoparticles. **Oral presentation at the 24th ASMR NSW Annual Scientific Meeting (6th June), Sydney, Australia**
- Heravi Shargh V, Hondermarck H, Liang, M. (2015) Albumin-dextran-chitosan hybrid nanoparticle enhances the efficacy of tyrosine kinase inhibitor GNF-5837 in breast cancer. **Poster presentation at the 4th NanoToday Conference (6-10th December), Dubai, United Arab Emirates**
- Heravi Shargh V, Hondermarck H, Liang, M. (2015) Enhancing the efficacy of tyrosine kinase inhibitors through bio-polymeric albumin hybrid nanoparticles in breast cancer. **Poster presentation at the Hunter Cancer Research Symposium (27th November), Hunter Medical Research Institute (HMRI), Newcastle, Australia**
- Heravi Shargh V, Hondermarck H, Liang, M. (2014) Multifunctional nanomedicines based on albumin for targeted breast cancer therapy. **Poster presentation at the Hunter Cancer Research Symposium (21st November), Hunter Medical Research Institute (HMRI), Newcastle, Australia**

List of Abbreviations

Ab	antibody
Alb	albumin
Alb-HNP	albumin hybrid nanoparticle
ATP	adenosine triphosphate
BCA	bicinchoninic acid
BSA	bovine serum albumin
CS	chitosan
DLS	dynamic light scattering
DMSO	dimethyl sulfoxide
DS	dextran sulphate
EDC	1-ethyl-3-(3-dimethylaminopropyl)carbodiimide
EPR	enhanced permeability and retention
FBS	foetal bovine serum
FDA	food and drug administration
Gel-Alb HNP	gelatine-albumin hybrid nanoparticle
GP	glycoprotein
HER-2	human epidermal growth factor receptor-2
HNPs	hybrid nanoparticles
HPLC	high-performance liquid chromatography
HSA	human serum albumin
IC_{50}	half-maximal inhibitory concentration
IgM	immunoglobulin M
MAPK	mitogen-activated protein kinase
MMP-2	matrix metalloproteinase-2
MTD	maximum tolerated dose
MW	molecular weight
Nab™	nanoparticle albumin-bound technology

NGF	nerve growth factor
NPs	nanoparticles
PBS	phosphate buffered saline
PEC	polyelectrolyte complex
PEG	polyethylene glycol
Pgp	P-glycoprotein
RES	reticuloendothelial system
RTKs	receptor tyrosine kinases
SEM	scanning electron microscope
SPARC	secreted protein, acidic and rich in cysteine
TrkA	tropomyosin receptor kinase A

Thesis Overview

In this thesis, our aim was to develop novel albumin hybrid NPs (Alb-HNPs) that not only enable tumour accumulation and site-specific drug release in tumour microenvironment, but also exploit the natural trafficking of albumins to enhance the uptake of the bound drug by cancer cells. Furthermore, this thesis is the first known attempt to investigate the anti-cancer effects of the selective TrkA inhibitor GNF-5837 in breast cancer.

This thesis has been divided into 5 chapters:

Chapter 1 is an introduction that summarises the established body of knowledge related to the research topics in this thesis. It gives some general background on the use of nanotechnology in cancer therapy and introduces different types of nanocarriers for cancer drug delivery. It then focuses on the current progress and development of albumin-based nanocarriers. Later, it introduces the involvement of TrkA signalling in breast cancer and the potential of TrkA inhibitors as molecularly-targeted therapeutics. Finally, our research aims and hypotheses are defined in the end of this chapter.

Chapters 2 and 3 are two original research papers that result from the research project. Chapter 2 involves the development of novel Alb-HNPs that enhanced the targeting specificity and efficacy of TrkA inhibitor against breast cancer cells. To continue with that work, Chapter 3 describes the further development of gelatine-albumin HNPs (Gel-Alb HNPs) that facilitate cellular uptake of TrkA inhibitor through site-specific size reduction and drug release in the tumour microenvironment. Each paper includes the materials and methods used in that study as well as the presentation and discussion of research results. A linking text before each paper has been provided to state the context and rationale for the work presented in the paper and situate the paper in the context of the research project as a whole.

Chapter 4 includes the general summary, discussions and conclusions of the thesis. Chapter 5 is our published review paper that discusses the important considerations for the design of antibody-targeted nanoparticles for cancer therapy. It serves as the relevant future perspective of this thesis for the development of next generation Alb-HNPs with active targeting to further enhance targeting efficiency and improve therapeutic outcomes.