UNIVERSITI PUTRA MALAYSIA

COMBINATION THERAPY USING PLASMID DNA-MEDIATED VP3 AND SHCD147 GENES FOR COLON CANCER IN A MURINE MODEL

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By

RUZILA ISMAIL

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirement for the Degree of Doctor of Philosophy

December 2014

Seeking His pleasure...

Dedicated to my father Ismail Mohamed,

My late mother Pathimah Md.Nasir,

My husband Ahmad Mubarak Tajul Arifin

and my beloved family

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

COMBINATION THERAPY USING PLASMID DNA-MEDIATED VP3 AND SHCD147 GENES FOR COLON CANCER IN A MURINE MODEL

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Chairman : Associate Professor Zeenathul Nazariah Allaudin, PhD

Faculty : Institute of Bioscience

Several strategies have been pursued to develop cancer therapies that selectively act on and kill cancer cells preferentially, leaving normal cells unaffected. The VP3, an avian virus-derived protein, can specifically impulse the death signal to different signal transduction pathways and finally lead to the apoptosis of the host cancer cells. The present work utilized a stress-inducing promoter which is a glucose-regulated protein (GRP) promoter to enhance VP3 expression in solid tumor condition. On the other hand, the over-expression of Basigin/CD147, a transmembrane glycoprotein has association with malignant melanoma invasiveness, metastasis and angiogenesis. Since CD147 has been indicated to be one of the critical cell-surface proteins in promoting chemo-resistance and survivability of cancer stem cells (CSC), down regulation of its expression by RNAi is an attractive way to suppress CD147-dependent cell proliferation, invasion and metastatic activity of cancer cells to eventually induce anoikis. All necessary impact of efficiencies and *in vitro* evaluation to optimize in this project has been established. In this study, tumor-bearing murine model was established. Investigation was conducted on 175-200 mm^3 tumor model receiving highly purified plasmid DNA in single treatment or combination of VP3 and shCD147 via intratumoral route (n=8). An alternate-date dosing approach was practiced whenever 3 doses were needed. Control groups were either a) non-treated, b) received 3 doses of 100 µg of pVIVO1-GFP/LacZ or c) 3 doses of 100 µg of psiRNA-h7SKzeo. Whilst treated mice received either a) 3 doses of 100 µg of pVIVO1-GFP/VP3 or b) 3 doses of 100 µg of psiRNA-CD147. For combinative therapy, mice received either a) 3 doses of 50 µg of pVIVO1-GFP/VP3 with combination of 3 doses of 50 µg of psiRNA-CD147 or b) 3 doses of 100 µg of pVIVO1-GFP/VP3 plus 3 doses of 100 µg of psiRNA-CD147, as representative for low-dose and high-dose respectively. All the 56 mice were subjected to 12 h light/ 12 h dark cycle and kept in individually ventilated cages (IVC) with constant rotation rate of 70 air-changes/ h to ensure sterility. Administration of VP3 alone led to

percentage tumor growth inhibition (TGI) of 40.0% and a 1.3-fold increase in the tumor growth delay index (TGDI) whilst administration of shCD147 led to TGI of 45.2% and 1.2-fold increase in the TGDI value. Whereas combination of low-dose treatment led to TGI of 51.1% ($p<0.001$) and a 2.0-fold increase in the TGDI whilst high-dose combinative treatment led to higher TGI of 60.3% ($p<0.001$) and 2.3-fold increase in the TGDI. These results demonstrated an extensive inhibition of CT26 tumor xenograft growth by VP3 and shCD147 combinative approach. Moreover, there is no discernible effect observed on histopathology and clinical chemistry profile of the host. The anti-proliferation and anti-angiogenic activities of the combinative approach were investigated by the use of the immunohistochemistry analysis, PCNA and vWF. Apoptotic cells were determined in the treated group by DNA fragmentation, TUNEL assay and confirmed by AnnV/PI double staining. The apoptosis percentage in the combinatively (VP3 + shCD14/2) treated tumor was markedly increased compared to individually treated samples at day-25 post-treatment. Here, it was found that CD147 silencing induced dual coordinated effects, resulting in inhibition of tumor cell proliferation and sensitization to VP3-induced apoptosis. In conclusion, this study showed that the combinative approach was more promising and effective in controlling tumor growth and inducing apoptosis than introducing VP3 or shCD147/2 alone. The combinative treatment also offers potential advantages in control of tumorigenesis, and thus deserves further research as a preferred approach in cancer gene therapy.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

TERAPI GABUNGAN MENGGUNAKAN GEN VP3 DAN SHCD147 BERPERANTARAAN DNA PLASMID UNTUK KANSER KOLON DALAM MODEL MENCIT

Oleh

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Disember 2014

Pengerusi : Profesor Madya Zeenathul Nazariah Allaudin, PhD

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Beberapa strategi telah dilaksanakan untuk membangunkan terapi kanser yang bertindak secara terpilih dan membunuh sel-sel kanser secara pilihan, meninggalkan sel-sel normal tidak terjejas. VP3, protein virus yang diperolehi dari burung, boleh mendorong isyarat kematian yang berbeza isyarat transduksi dan akhirnya membawa kepada apoptosis kepada sel-sel kanser perumah secara khusus. Kajian semasa menggunakan promotor tekanan-mendorong iaitu promotor glukosa dikawal-selia protein (GRP) untuk meningkatkan ekspresi VP3 dalam keadaan tumor pepejal. Di sisi lain, lebih-ekspresi Basigin/CD147, glikoprotein transmembran mempunyai kaitan dengan pencerobohan, metastatik dan angiogenik melanoma malignan. Memandangkan CD147 telah ditunjukkan untuk menjadi salah satu protein sel permukaan kritikal dalam menggalakkan rintangan kimo dan kemandirian stem sel kanser (CSC), menurunkan ekspresinya dengan RNAi adalah cara yang menarik untuk menindas proliferasi sel, serangan dan aktiviti metastatik sel-sel kanser yang bergantung kepada CD147 untuk akhirnya mendorong kepada anoikis. Semua kesan kecekapan dan penilaian dalam vitro yang perlu untuk mengoptimumkan dalam projek ini telah dilakukan. Dalam kajian ini, model mencit pembawa-tumor telah ditubuhkan. Siasatan telah dijalankan ke atas model tumor 175-200 mm^3 yang menerima plasmid DNA berketulenan tinggi dalam rawatan tunggal atau kombinasi VP3 dan shCD147/2 melalui kaedah intratumoral (n=8). Pendekatan dos silih ganti hari diamalkan apabila 3 dos yang diperlukan. Kumpulan kawalan adalah sama ada a) tidak dirawat, b) menerima 3 dos 100 μg daripada pVIVO1-GFP/LacZ atau c) 3 dos 100 μg daripada psiRNA-h7SKzeo. Manakala mencit yang dirawat menerima sama ada a) 3 dos 100 μg daripada pVIVO1-GFP/VP3 atau b) 3 dos 100 μg daripada psiRNA-CD147/2. Untuk terapi kombinasi, mencit menerima sama ada a) 3 dos 50 μg daripada pVIVO1-GFP/VP3 dengan gabungan 3 dos 50 μg daripada psiRNA-CD147/2 atau b) 3 dos 100 μg daripada pVIVO1-GFP/VP3 ditambah 3 dos 100 μg

daripada psiRNA-CD147/2, masing-masing sebagai wakil dos rendah dan dos tinggi. Kesemua 56 mencit telah tertakluk kepada kitaran 12 jam cahaya/12 jam gelap dan disimpan dalam sangkar pengudaraan secara individu (IVC) dengan kadar putaran udara berterusan 70 kali/jam untuk memastikan kesterilan. Pemberian suntikan VP3 membawa kepada perencatan peratusan pertumbuhan tumor (TGI) sebanyak 40.0% dan peningkatan 1.3 kali ganda dalam indeks kelewatan pertumbuhan tumor (TGDI) manakala pemberian suntikan shCD147/2 menyebabkan TGI sebanyak 45.2% dan peningkatan 1.2 kali ganda dalam nilai TGDI. Sedangkan gabungan rawatan dos rendah menyebabkan TGI sebanyak 51.1% ($p < 0.001$) dan peningkatan 2.0 kali ganda dalam TGDI manakala rawatan kombinasi dos tinggi membawa kepada yang lebih tinggi TGI sebanyak 60.3% ($p < 0.001$) dan peningkatan 2.3 kali ganda dalam TGDI. Keputusan ini menunjukkan satu perencatan yang banyak terhadap pertumbuhan tumor xenograft CT26 oleh pendekatan kombinasi VP3 dan shCD147. Tambahan pula, tidak ada kesan yang ketara diperhatikan pada histopatologi dan profil kimia klinikal perumah. Aktiviti anti-proliferasi dan anti-angiogenik daripada pendekatan kombinasi telah disiasat oleh penggunaan analisis immunohistokimia, PCNA dan vWF. Sel-sel apoptotic ditentukan dalam kumpulan yang dirawat menggunakan fragmentasi DNA, asei TUNEL dan disahkan menggunakan pewarnaan berganda AnnV/PI. Peratusan apoptosis dalam tumor dirawat secara kombinasi (VP3 + shCD14/2) telah meningkat dengan ketara berbanding dengan sampel yang dirawat secara individu pada hari-25 selepas rawatan. Di sini, didapati bahawa penyenyap CD147 mendorong kesan penyelarasan dwi, menyebabkan perencatan percambahan sel tumor dan pemekaan untuk apoptosis VP3-teraruh. Kesimpulannya, kajian ini menunjukkan bahawa pendekatan secara kombinasi lebih menjanjikan dan berkesan dalam mengawal pertumbuhan tumor dan mendorong apoptosis daripada memperkenalkan VP3 atau shCD147/2 sahaja. Rawatan secara kombinasi juga menawarkan kelebihan yang berpotensi mengawal tumorigenesis, dan dengan itu layak mendapat penyelidikan lanjut sebagai pendekatan utama dalam terapi gen kanser.

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I certify that a Thesis Examination Committee has met on 8 December 2014 to conduct the final examination of Ruzila Ismail on her thesis entitled "Combination Therapy using Plasmid DNA-Mediated VP3 and shCD147 Genes for Colon Cancer in a Murine Model" in accordance with Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the degree of Doctor of Philosophy.

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LIST OF ABBREVIATIONS

%	Percentage
µl	microliter
A	ampere
a.a	Amino acid
AI	Apoptotic Index
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
APS	Ammonium persulfate
AST	Aspartate aminotransferase
ATV	Antibiotic Trypsin Versin
BLAST	Basic Local Alignment Search Tool
bp	Base pair
BSA	Bovine serum albumin
BUN	Blood urea nitrogen
CAV	Chicken anaemia virus
CD147	Cluster of differentiation 147
CMVenh	Cytomegalovirus enhancer
CRC	Colorectal cancer
CT26	Colon Tumor #26
Cyp	cyclophilin
DAB	3, 3'-diaminobenzidine
ddH_2O	deionised distilled water
dH_2O	distilled water
DNA	Deoxyribonucleic Acid
dNTPs	Dideoxynucleotide triphosphates (dATP, dTTP, dCTP and dGTP)
dsRNA	Double-stranded RNA
E. coli	Escherichia coli
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme Linked Immunosorbent Assay
EMMPRIN	Extracellular matrix metalloproteinase inducer
et al.	et alii
EtBr	Ethidium bromide
FBS	Fatal bivine serum
FITC	Fluorescein isothiocyanate
g	Gram
G	Gauge
GFP	Green fluorescent protein
GRP	Glucose regulate protein
h	Hour(s)
HIV	Human Immunodeficiency Virus
HSV	Herpes Simplex Virus
i.e.	"id est"

i.m	Intramuscular
i.p	Intraperitoneal
i.t	Intratumoral
i.v	Intravenous
Ig	immunoglobulin
IHC	Immunohistochemistry
Kb	Kilo base
kDA	Kilo Dalton
mA	Milliampere
MCT	monocarboxylate transporter
mg	Milligram
$MgCl_2$	Magnesium chloride
min	minute
miRNA	micro Ribonucleic Acid
ml	milliliter
mM	millimolar
MMP	Matrix metalloproteinase
mRNA	Messenger Ribonucleic Acid
MT1	membrane type 1
MTT	3-(4,-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide
ng	nanogram
nt	Nucleotide(s)
NTC	Non-template control
PBS	Phosphate buffered saline
PBST	Phosphate buffered saline tween-20
PCD	Program cell death
PCNA	Proliferating Cell Nuclear Antigen
PCR	Polymerase chain reaction
pol	polymerase
PVDF	Polyvinylidene difluoride
RA	rheumatoid arthritis
RE	Restriction endonuclease
RISC	RNA Interference Specificity Complex
RNA	Ribonucleic Acid
RNAi	Ribonucleic Acid interference
rpm	Revolution per minute
RPMI	Roswell Park Memorial Institute medium
RT	Reverse transcription or reverse transcriptase
RTV	Relative tumor volume
s.c.	Subcutaneous
SDS	Sodium Dodecyl Sulfate
SDS-PAGE	SDS-polyacrylamide gel electrophoresis
sec	Second(s)

shRNA	Short hairpin Ribonucleic Acid
siRNA	Short interfering Ribonucleic Acid
ssRNA	Single-stranded Ribonucleic Acid
SV40enh	Simian virus 40 enhancer
TAE	Tris acetate EDTA buffer
Taq	Thermus aquaticus thermostable DNA
TBE	Tris borate EDTA buffer
TCSF	Tumor collagenase stimulatory factor
TE	Tris-EDTA buffer
TEMED	N, N, N', N'-tetramethylethylenediamine
TGD	Tumor growth delay
TGDI	Tumor growth delay index
TGI	Tumor growth inhibition
TIMP	Tissue inhibitor of metalloproteinase
TUNEL	Terminal deoxynucleotidyltransferse-mediated dUTP Nick End Labeling
UV	Ultraviolet
V	Volt
VP3	Virus protein 3
vWF	von Willebrand factor

CHAPTER 1

INTRODUCTION

1.1 Background

Cancer remains one of mankind's most feared diseases worldwide. Malignant cancer has remained as one of the five main causes of mortality for the past 20 years. According to International Agency Research for Cancer, colorectal cancer was the third most commonly diagnosed cancer worldwide with total of 1.36 million of incidence in 2012 (IARC 2014a). In Malaysia, cancer incidence was listed as one of the ten leading causes of hospitalization (3.82%) and five leading causes of death (13.63%) in both Ministry of Health (MoH) and private hospitals (MoH, 2013). Colorectal cancer was the second commonest form of cancer in both Malaysian men and women after lung and breast cancer, respectively. According to National Cancer Registry, a total of 1185 (14.6%) of male and 1011 (10.0%) of female incidence per 100,000 population were reported in 2007 and the figure was expected to increase (Omar & Ibrahim Tamin, 2011).

High incidence in colorectal cancer was due to the delay in diagnosis and ineffectiveness in treatment. The current treatment applies begin with surgical procedures to excise colon tumor segments followed by radiotherapy and/or chemotherapy. These conventional treatments may not be effective as it is not only killing the cancer cells, but also the healthy cells surround. Besides, their adverse side-effects are debilitating for patients. Thus, it is crucial to develop new therapeutic methods for cancer so that cancer patients can have more alternative for the best therapy they need.

Gene therapy is an alternative method to cure or slow down the progression of advanced stage of malignant cancer. In somatic gene therapy, therapeutic DNA transgene either integrated in the genome, as an external episomal or plasmid are introduce into cells. Selective therapy for cancer cells, only attacking actively dividing cells while sparing normal cells unaffected is an emerging field in recent years. Several viruses need actively dividing cells during completion of their life cycle, thus viral proteins have gained attention as a feasible cancer selective therapy.

Apoptosis or programmed cell death is an energy-dependent, innate and genetically determined process utilized by multicellular organisms to eliminate unwanted or damaged cells. Cancer is one of the diseases that is related to the loss of control between apoptosis and survival (Bjelaković et al., 2005). The use of apoptosis inducing gene is one of the alternative treatments for cancer therapy. Ideally, the killing cancer cells via apoptosis is a safer way for cancer therapy because the side effects of conventional treatments can be reduced to a minimum and the choice of

targeting therapeutic gene can kill cancer cells without affecting the surrounding cells.

VP3, a product of the third open reading frame (ORF) of the chicken anaemia virus can specifically impulse the apoptosis signals in cancer cells (Backendorf et al., 2008). Not hampered by tumor-suppressor p53 mutations or over-expression of anti-apoptosis proteins makes VP3 the most eligible candidate for various cancers. Therefore, recombinant plasmid expressing the VP3 therapeutic gene will increase chances of cure for cancer patient.

In addition, numerous macromolecules and cellular agents have been attempted to target tumor cells more specifically rather than other factors related to tumor growth. These include cytokines, small molecules antagonists, monoclonal antibodies, oligonucleotides and gene-targeting vector (Wong, 2011). Although apoptosis is a popular goal in treatment strategies, current preclinical and clinical findings have demonstrated the lack of desired expectation in achieving this matter. This is due to evasion of apoptosis by cancer cells.

These disappointing results arise due to multiple factors predominantly the vasculatures that supply nutrients and oxygen in tumors. When the continuous supply of nutrients and oxygen to the tumor cells keeps tumors growing, even well-designed drugs are hindered from acting on the target in an effective manner. One promising solution arisen from the observation that growing tumors are dependent on the multiple vascularisation mechanism, angiogenesis (Döme et al., 2007).

CD147, an integral plasma membrane protein has the capability to promote angiogenesis formation through stimulation of vascular endothelial growth factor (VEGF) expression on cancer cells (Tang et al., 2005). Not restricted to VEGF, it stimulates abundance of matrix metalloproteinase (MMP)-production which cause extracellular matrix degradation and increased cellular migration and invasion. During metastases, usually majority of cells undergo anoikis, a kind of apoptosis which is induced by the detachment of anchorage-dependent cells from the adjacent extracellular matrix. However, CD147 was identified as a contributor to anoikis-resistance which leads to tumor cells metastases (Ke et al., 2012). Recently, CD147 has been indicated to be one of the critical cell-surface proteins in promoting chemo-resistant and survivability of cancer stem cells (CSC) (Kang et al., 2013). In this regard, down regulation of its expression using RNA interference is an attractive way to suppress CD147-dependent cell proliferation, invasion and metastases activity of cancer cells to eventually induce anoikis.

Before entering into human clinical trials, the safety of the incipient therapeutic gene has to be substantiated with animal studies. As consideration to safety in gene therapy, it is customary to assess the biodistribution of the DNA plasmid in order to cater information on potential toxicities. The development of new, safe and effective cancer therapy for 21st century are at the midst of explosion. Scientists have achieved

an advance experimental in the simultaneous use of two or more agents for treating cancer. However, new technologies in combination therapy need innovative approaches, new models, standards and assays.

The aim of this study is to understand the effect and interaction between the tumor and its blood vessel during combinatorial pro-apoptotic and anti-tumorigenic therapy. Recently developed genomic and proteomic technologies, gene silencing against CD147 will be applied as anti-tumorigenic therapy. Due to short half-life of the siRNA, here, gene silencing will be applied in a vector form for long term expression. Anti-tumorigenic agent would be expected to inhibit tumor cell proliferation and invasion, while enhancing sensitization to pro-apoptotic agent induced apoptosis. In this study, tumors will be treated with combined pro-apoptotic and anti-tumorigenic therapy. This combinative approach may improve effectiveness of the tumor treatment.

1.2 Hypothesis of This Study

It is hypothesized that by utilizing inducible GRP78 promoter to drive cancer-selective VP3 expression would improve the expression in solid tumor while combinative approach of pro-apoptotic and anti-tumorigenic gene therapy would provide better and more effective means in treating and inducing apoptosis in tumor-bearing model.

1.3 Objectives of This Study

This study investigates the effects and efficiency of plasmid DNA-mediated VP3 and shCD147 genes therapy in murine model for colon cancer including:

1) To develop recombinant plasmids harboring VP3 (pVIVO1-GFP/VP3) and shCD147 (psiRNA-CD147).

2) To compare between GRP78 and CMV driven VP3 expression *in vitro.*

3) To select the best shCD147 construct with highest silencing effect *in vitro*.

4) To evaluate *in vivo* biodistribution and acute toxicity effect of pVIVO1-GFP/VP3 using effective dose and up to 4X effective dose.

5) To investigate the inhibition of tumor growth and apoptosis analysis of VP3, shCD147/2 or combination treatment in tumor-bearing murine model.

6) To examine the effect of VP3, shCD147/2 or combination treatments against cell proliferation and invasion in post-treated tumor mass.

REFERENCES

Aagaard, L., & Rossi, J. J. (2008). RNAi therapeutics: principles, prospects and challenges. *Advanced Drug Delivery Reviews*, *59*, 75–86.

Abraham, D., Zins, K., Sioud, M., Lucas, T., & Aharinejad, S. (2008). Host CD147 blockade by small interfering RNAs suppresses growth of human colon cancer xenografts. *Frontiers in Bioscience*, *13*, 5571–5579.

Aharinejad, S., Paulus, P., Sioud, M., Hofmann, M., Zins, K., Schafer, R., Stanley, E. R., & Abraham, D. (2004). Colony-stimulating Factor-1 blockade by antisense oligonucleotides and small interfering RNAs suppresses growth of hhuman mammary tumor xenografts in mice. *Cancer Research*, *64*, 5378–5384.

Akinci, S. B., Ulu, N., Yondem, O. Z., Firat, P., Guc, M. O., Kanbak, M., & Aypar, U. (2005). Effect of neostigmine on organ injury in murine endotoxemia: missing facts about the cholinergic antiinflammatory pathway. *World Journal of Surgery*, *29*, 1483–1489.

Aliabadi, H. M., Landry, B., Sun, C., Tang, T., & Uludağ, H. (2012). Supramolecular assemblies in functional siRNA delivery: where do we stand? *Biomaterials*, *33*, 2546–2569.

Allain, F., Vanpouille, C., Carpentier, M., Slomianny, M.-C., Durieux, S., & Spik, G. (2002). Interaction with glycosaminoglycans is required for cyclophilin B to trigger integrin-mediated adhesion of peripheral blood T lymphocytes to extracellular matrix. *Proceedings of the National Academy of Sciences of the United States of America*, *99*, 2714–2719.

Altruda, F., Cervella, P., Gaeta, M. L., Daniele, A., Giancotti, F., Tarone, G., Stefanuto, G., & Silengo, L. (1989). Cloning of cDNA for a novel mouse membrane glycoprotein (gp42): shared identity to histocompatibility antigens, immunoglobulins and neural-cell adhesion molecules. *Gene*, *85*, 445–451.

Amarzguioui, M., Rossi, J., & Kim, D. (2005). Approaches for chemically synthesized siRNA and vector-mediated RNAi. *FEBS Letters*, *579*, 5974–5981.

Arjumand, W., Seth, A., & Sultana, S. (2011). Rutin attenuates cisplatin induced renal inflammation and apoptosis by reducing NFκB, TNF-α and caspase-3 expression in wistar rats. *Food and Chemical Toxicology*, *49*, 2013–2021. Elsevier Ltd.

Armengol, G., Ruiz, L. M., & Orduz, S. (2004). The injection of plasmid DNA in mouse muscle results in lifelong persistence of DNA, gene expression, and humoral response. *Molecular Biotechnology*, *27*, 109–118.

Arroyo, J. D., Gallichotte, E. N., & Tewari, M. (2014). Systematic design and functional analysis of artificial microRNAs. *Nucleic Acids Research*, *2*, 1–14.

Backendorf, C., Visser, A. E., De Boer, A. G., Zimmerman, R., Visser, M., Voskamp, P., Zhang, Y.-H., & Noteborn, M. (2008). Apoptin: therapeutic potential of an early sensor of carcinogenic transformation. *Annual Review of Pharmacology and Toxicology*, *48*, 143–169.

Balaji, N., Santha Devy, A., Sumathi, M. K., Vidyalakshmi, S., Sathish Kumar, G., & D'Silva, S. (2013). Annexin v - affinity assay - apoptosis detection system in granular cell ameloblastoma. *Journal of International Oral Health*, *5*, 25–30.

Belton, R. J., Chen, L., Mesquita, F. S., & Nowak, R. A. (2008). Basigin-2 is a cell surface receptor for soluble basigin ligand. *The Journal of Biological Chemistry*, *283*, 17805–17814.

Bish, L. T., Sleeper, M. M., Reynolds, C., Gazzara, J., Withnall, E., Singletary, G. E., Buchlis, G., Hui, D., High, K. A., Gao, G., Wilson, J. M., & Sweeney, H. L. (2011). Cardiac gene transfer of short hairpin RNA directed against phospholamban effectively knocks down gene expression but causes cellular toxicity in canines. *Human Gene Therapy*, *22*, 969–977.

Biswas, C., Zhang, Y., DeCastro, R., Guo, H., Nakamura, T., Kataoka, H., & Nabeshima, K. (1995). The human tumor cell-derived collagenase stimulatory factor (renamed EMMPRIN) is a member of the immunoglobulin superfamily. *Cancer Research*, *55*, 434–439.

Bjelaković, G., Nagorni, A., Bjelaković, M., Stamenković, I., Arsić, R., & Katić, V. (2005). Apotosis: Programmed cell death and its clinical implications. *Medicine and Biology*, *12*, 6–11.

Boden, D., Pusch, O., Silbermann, R., Lee, F., Tucker, L., & Ramratnam, B. (2004). Enhanced gene silencing of HIV-1 specific siRNA using microRNA designed hairpins. *Nucleic Acids Research*, *32*, 1154–1158.

Bordador, L. C., Li, X., Toole, B., Chen, B., Regezi, J., Zardi, L., Hu, Y., & Ramos, D. M. (2000). Expression of emmprin by oral squamous cell carcinoma. *International Journal of Cancer*, *85*, 347–352.

Boudreau, R. L., Martins, I., & Davidson, B. L. (2009). Artificial microRNAs as siRNA shuttles: improved safety as compared to shRNAs in vitro and in vivo. *Molecular Therapy*, *17*, 169–175.

Boudreau, R. L., Monteys, A. M., & Davidson, B. L. (2008). Minimizing variables among hairpin-based RNAi vectors reveals the potency of shRNAs. *RNA*, *14*, 1834–1844.

Boye, K., Nesland, J. M., Sandstad, B., Haugland Haugen, M., Mælandsmo, G. M., & Flatmark, K. (2012). EMMPRIN is associated with S100A4 and predicts patient outcome in colorectal cancer. *British Journal of Cancer*, *107*, 667–674.

Brennan, F. R., & Dougan, G. (2005). Non-clinical safety evaluation of novel vaccines and adjuvants: New products, new strategies. *Vaccine*, *23*, 3210–3222.

Brenner, H., Kloor, M., & Pox, C. P. (2014). Colorectal cancer. *Lancet*, *383*, 1490–502.

Butash, K. A., Natarajan, P., Young, A., & Fox, D. K. (2000). Reexamination of the effect of endotoxin on cell proliferation and transfection efficiency. *BioTechniques*, *29*, 610–619.

Cadet, P., Mantione, K. J., & Stefano, G. B. (2003). Molecular Identification and Functional Expression of μ 3, a Novel Alternatively Spliced Variant of the Human μ Opiate Receptor Gene. *The Journal of Immunology*, *170*, 5118–5123.

Cai, S., Bulus, N., Fonseca-Siesser, P. M., Chen, D., Hanks, S. K., Pozzi, A., & Zent, R. (2005). CD98 modulates integrin beta1 function in polarized epithelial cells. *Journal of Cell Science*, *118*, 889–899.

Cai, X., Hagedorn, C. H., & Cullen, B. R. (2004). Human microRNAs are processed from capped, polyadenylated transcripts that can also function as mRNAs. *RNA*, *10*, 1957–1966.

Campbell, T. W. (2012). Chemical chemistry of mammals: laboratory animals and miscellaneous species. In M. A. Thrall, G. Weiser, R. W. Allison, & T. W. Campbell (Eds.), *Veterinary Hematology and Clinical Chemistry* (Second Edi., pp. 571–581). John Wiley & Sons, Inc.

Castanotto, D., & Rossi, J. J. (2009). The promises and pitfalls of RNA-interference-based therapeutics. *Nature*, *457*, 426–433.

Castle, J. C., Loewer, M., Boegel, S., de Graaf, J., Bender, C., Tadmor, A. D., Boisguerin, V., Bukur, T., Sorn, P., Paret, C., Diken, M., Kreiter, S., Türeci, Ö., & Sahin, U. (2014). Immunomic, genomic and transcriptomic characterization of CT26 colorectal carcinoma. *BMC genomics*, *15*, 190.

Center, S. A. (2007). Interpretation of liver enzymes. *Veterinary Clinics Small Animal*, *37*, 297–333.

Chang, C. J., Shih, W. L., Yu, F. L., Liao, M. H., & Liu, H. J. (2004). Apoptosis induced by bovine ephemeral fever virus. *Journal of Virological Methods*, *122*, 165–170.

Chen, C. C., Hwang, J., Ting, G., Tsengc, Y. L., Wang, S. J., & Whang Peng, J. (2007). Monitoring and quantitative assessment of tumor burden using in vivo bioluminescence imaging. *Nuclear Instruments and Methods in Physical Research*, *571*, 437–441.

Chen, X., Lin, J., Kanekura, T., Su, J., Lin, W., Xie, H., Wu, Y., Li, J., Chen, M., & Chang, J. (2006). A small interfering CD147-targeting RNA inhibited the proliferation, invasiveness, and metastatic activity of malignant melanoma. *Cancer Research*, *66*, 11323–11330.

Chen, X., Zhang, D., Dennert, G., Hung, G., & Lee, A. S. (2000). Eradication of murine mammary adenocarcinoma through HSVtk expression directed by the glucose-starvation inducible grp78 promoter. *Breast Cancer Research and Treatment*, *59*, 81–90.

Chendrimada, T. P., Gregory, R. I., Kumaraswamy, E., Cooch, N., Nishikura, K., & Shiekhattar, R. (2010). TRBP recruits the Dicer complex to Ago2 for microRNA processing and gene silencing. *Nature*, *436*, 740–744.

Cheon, D. J., & Orsulic, S. (2011). Mouse models of cancer. *Annual Review of Pathology*, *6*, 95–119.

Cogoni, C., Irelan, J. T., Schumacher, M., Schmidhauser, T. J., Selkerl, E. U., & Macino, G. (1996). Transgene silencing of the al-1 gene in vegetative cells of Neurospora is mediated by a cytoplasmic effector and does not depend on DNA-DNA interactions or DNA methylation. *The EMBO Journal*, *1*, 3153–3163.

Coussens, L. M., Fingleton, B., & Matrisian, L. M. (2002). Matrix metalloproteinase inhibitors and cancer: trials and tribulations. *Science*, *295*, 2387–2392.

Cross, D., & Burmester, J. (2006). Gene therapy for cancer treatment: past, present and future. *Clinical medicine & research*, *4*, 218–27.

Cunningham, C. C., Chada, S., Merritt, J. A., Tong, A., Senzer, N., Zhang, Y., Mhashilkar, A., Parker, K., Vukelja, S., Richards, D., Hood, J., Coffee, K., & Nemunaitis, J. (2005). Clinical and local biological effects of an intratumoral injection of mda-7 (IL24; INGN 241) in patients with advanced carcinoma: A phase I study. *Molecular Therapy*, *11*, 149–59.

Cunningham, D., Atkin, W., Lenz, H. J., Lynch, H. T., Minsky, B., Nordlinger, B., & Starling, N. (2010). Colorectal cancer. *Lancet*, *375*, 1030–1047.

Curtin, K. D., Meinertzhagen, I. A., & Wyman, R. J. (2005). Basigin (EMMPRIN/CD147) interacts with integrin to affect cellular architecture. *Journal of Cell Science*, *118*, 2649–2660.

Dalal, S., Berry, A. M., Cullinane, C. J., Mangham, D. C., Grimer, R., Lewis, I. J., Johnston, C., Laurence, V., & Burchill, S. A. (2005). Vascular Endothelial Growth Factor: A Therapeutic Target for Tumors of the Ewing ' s Sarcoma Family. *Clinical Cancer Research*, *11*, 2364–2378.

Damsker, J. M., Okwumabua, I., Pushkarsky, T., Arora, K., Bukrinsky, M. I., & Constant, S. L. (2009). Targeting the chemotactic function of CD147 reduces collagen-induced arthritis. *Immunology*, *126*, 55–62.

Danen-Van Oorschot, A. A. A. M., Fischer, D. F., Grimbergen, J. M., Klein, B., Zhuang, S.-M., Falkenburg, J. H. F., Backendorf, C., Quax, P. H. A., Van Der Eb, A. J., & Noteborn, M. H. M. (1997). Apoptin induces apoptosis in human

transformed and malignant cells but not in normal cells. *Proceedings of the National Academy of Sciences of the United States of America*, *94*, 5843–5847.

Davies, R. J., Freeman, A., Morris, L. S., Bingham, S., Dilworth, S., Scott, I., Laskey, R. A., Miller, R., & Coleman, N. (2002). Analysis of minichromosome maintenance proteins as a novel method for detection of colorectal cancer in stool. *Lancet*, *359*, 1917–1919.

DeCastro, R., Zhang, Y., Guo, H., Kataoka, H., Gordon, M. K., Toole, B. p, & Biswas, G. (1996). Human keratinocytes express EMMPRIN, an extracellular matrix metalloproteinase inducer. *The Journal of Investigative Dermatology*, *106*, 1260–1265.

Delépine, P., Guillaume, C., Montier, T., Clément, J. C., Yaouanc, J. J., Des Abbayes, H., Berthou, F., Le Pape, A., & Férec, C. (2003). Biodistribution study of phosphonolipids: A class of non-viral vectors efficient in mice lung-directed gene transfer. *Journal of Gene Medicine*, *5*, 600–608.

Desjardins, L. M., & MacManus, J. P. (1995). An adherent cell model to study different stage of apoptosis. *Experimental Cell Research*, *216*, 380–387.

Dharmapuri, S., Peruzzi, D., Marra, E., Palombo, F., Bett, A. J., Bartz, S. R., Yong, M., Ciliberto, G., La Monica, N., Buser, C. A., Toniatti, C., & Aurisicchio, L. (2011). Intratumor RNA interference of cell cycle genes slows down tumor progression. *Gene Therapy*, *18*, 727–733.

Döme, B., Hendrix, M. J. C., Paku, S., Tóvári, J., & Tímár, J. (2007). Alternative vascularization mechanisms in cancer: Pathology and therapeutic implications. *The American Journal of Pathology*, *170*, 1–15.

Du, Z. M., Hu, C. F., Shao, Q., Huang, M. Y., Kou, C. W., Zhu, X. F., Zeng, Y. X., & Shao, J. Y. (2009). Upregulation of caveolin-1 and CD147 expression in nasopharyngeal carcinoma enhanced tumor cell migration and correlated with poor prognosis of the patients. *International Journal of Cancer*, *125*, 1832–1841.

Dykxhoorn, D. M., Novina, C. D., & Sharp, P. A. (2003). Killing the messenger: short RNAs that silence gene expression. *Nature Reviews. Molecular Cell Biology*, *4*, 457–467.

Dziegiel, P., Forgacz, J., Suder, E., & Surowiak, P. (2003). Prognostic significance of metallothionein expression in correlation with Ki-67 expression in adenocarcinomas of large intestine. *Histology and Histopathology*, *18*, 401–407.

Van der Eb, M. M., Pietersen, A. M., Speetjens, F. M., Kuppen, P. J. K., Van De Velde, C. J. H., Noteborn, M. H. M., & Hoeben, R. C. (2002). Gene therapy with apoptin induces regression of xenografted human hepatomas. *Cancer Gene Therapy*, *9*, 53–61.

Egawa, N., Koshikawa, N., Tomari, T., Nabeshima, K., Isobe, T., & Seiki, M. (2006). Membrane type 1 matrix metalloproteinase (MT1-MMP/MMP-14) cleaves and releases a 22-kDa extracellular matrix metalloproteinase inducer (EMMPRIN) fragment from tumor cells. *The Journal of Biological Chemistry*, *281*, 37576–37585.

Ejima, K., Koji, T., Tsuruta, D., Nanri, H., Kashimura, M., & Ikeda, M. (2000). Induction of Apoptosis in Placentas of Pregnant Mice Exposed to Lipopolysaccharides: Possible Involvement of Fas/Fas Ligand System. *Biology of Reproduction*, *62*, 178–185.

Elbashir, S. M., Harborth, J., Lendeckel, W., Yalcin, A., Weber, K., & Tuschl, T. (2001). Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells. *Nature*, *411*, 494–498.

Elmore, S. (2007). Apoptosis: a review of programmed cell death. *Toxicologic Pathology*, *35*, 495–516.

Essers, J., Theil, A. F., Baldeyron, C., van Cappellen, W. A., Houtsmuller, Adriaan B. Kanaar, R., & Vermeulen, W. (2005). Nuclear dynamics of PCNA in DNA replication and repair. *Molecular and Cellular Biology*, *25*, 9350–9359.

Eun, S. K., Chae, Y. K., Seon, B. K., Se, J. I., Se, H. Y., Young, C. S., & Byong, M. K. (2007). In vivo kinetics and biodistribution of HB-110, a novel HBV DNA vaccine, after administration in mice. *Archives of Pharmacal Research*, *30*, 355–360.

Fadool, J. M., & Linser, P. J. (1993). 5A11 antigen is a cell recognition molecule which is involved in neuronal-glial interactions in avian neural retina. *Developmental Dynamics*, *196*, 252–262.

Fan, F., Schimming, A., Jaeger, D., & Podar, K. (2012). Targeting the tumor microenvironment: focus on angiogenesis. *Journal of Oncology*, *2012*, 281–261.

FDA. (2007). Guidance for industry: Considerations for plasmid DNA vaccines for infectious disease indications. *Biotechnology Law Report*, *26*, 641–648.

Fire, A., Xu, S., Montgomery, M. K., Kostas, S. A., Driver, S. E., & Mello, C. C. (1998). Potent and specific genetic interferenceby double-stranded RNA in Caenorhabditis elegans. *Nature*, *391*, 806–811.

Fossum, S., Mallett, S., & Barclay, A. N. (1991). The MRC OX-47 antigen is a member of the immunoglobulin superfamily with an unusual transmembrane sequence. *European Journal of Immunology*, *21*, 671–679.

Fushan, A. A., & Drayna, D. (2009). MALS: an efficient strategy for multiple site-directed mutagenesis employing a combination of DNA amplification, ligation and suppression PCR. *BMC Biotechnology*, *9*, 83.

Gabison, E. E., Hoang-Xuan, T., Mauviel, A., & Menashi, S. (2005). EMMPRIN/CD147, an MMP modulator in cancer, development and tissue repair. *Biochimie*, *87*, 361–368.

Gallagher, S. M., Castorino, J. J., & Philp, N. J. (2009). Interaction of monocarboxylate transporter 4 with beta1-integrin and its role in cell migration. *American Journal of Physiology. Cell Physiology*, *296*, C414–C421.

Gazit, G., Hung, G., Chen, X., Anderson, W. F., & Lee, A. S. (1999). Use of the glucose starvation-inducible glucose-regulated protein 78 promoter in suicide gene therapy of murine fibrosarcoma. *Cancer Research*, *59*, 3100–3106.

Gentry, B. G., Boucher, P. D., & Shewach, D. S. (2006). Hydroxyurea induces bystander cytotoxicity in cocultures of herpes simplex virus thymidine kinase-expressing and nonexpressing HeLa cells incubated with ganciclovir. *Cancer Research*, *66*, 3845–51.

Giering, J. C., Grimm, D., Storm, T. A., & Kay, M. A. (2008). Expression of shRNA from a tissue-specific pol II promoter is an effective and safe RNAi therapeutic. *Molecular Therapy*, *16*, 1630–1636.

Gilchuk, I., Irodov, D., Starokadomskyy, P., Pishel, I., Toporova, O., Novikova, S., & Kordium, V. (2006). The distribution of injected plasmid DNA throughout the organs and the expression of human apoA-I gene in vivo. *Biopolymers and Cell*, *22*, 439–445.

Gill, D. R., Smyth, S. E., Goddard, C. A., Pringle, I. A., Higgins, C. F., Colledge, W. H., & Hyde, S. C. (2001). Increased persistence of lung gene expression using plasmids containing the ubiquitin C or elongation factor 1alpha promoter. *Gene Therapy*, *8*, 1539–46.

Gorman, A. M., Szegezdi, E., Quigney, D. J., & Samali, A. (2005). Hsp27 inhibits 6-hydroxydopamine-induced cytochrome c release and apoptosis in PC12 cells. *Biochemical and Biophysical Research Communications*, *327*, 801–810.

Gravier, R., Dory, D., Laurentie, M., Bougeard, S., Cariolet, R., & Jestin, A. (2007). In vivo tissue distribution and kinetics of a pseudorabies virus plasmid DNA vaccine after intramuscular injection in swine. *Vaccine*, *25*, 6930–6938.

Gregory, R. I., Chendrimada, T. P., Cooch, N., & Shiekhattar, R. (2005). Human RISC couples microRNA biogenesis and posttranscriptional gene silencing. *Cell*, *123*, 631–640.

Greish, K. (2007). Enhanced permeability and retention of macromolecular drugs in solid tumors: A royal gate for targeted anticancer nanomedicines. *Journal of Drug Targeting*, *15*, 457–464.

Grimm, D. (2011). The dose can make the poison: lessons learned from adverse in vivo toxicities caused by RNAi overexpression. *Silence*, *2*, 1–6. BioMed Central Ltd.

Grimm, D., Streetz, K. L., Jopling, C. L., Storm, T. A., Pandey, K., Davis, C. R., Marion, P., Salazar, F., & Kay, M. A. (2006). Fatality in mice due to oversaturation of cellular microRNA/short hairpin RNA pathways. *Nature*, *441*, 537–541.

Griswold, D. P., & Corbett, T. H. (1975). A colon tumor model for anticancer agent evaluation. *Cancer*, *36*, 2441–2444.

Guan, G., Zhao, M., Liu, L., Jin, C., Sun, K., Zhang, D., Yu, D., Cao, H., Lu, Y., & Wen, L. (2013). Salmonella typhimurium Mediated Delivery of Apoptin in Human Laryngeal Cancer. *International Journal of Medical Sciences*, *10*, 1639–1648.

Guo, H., Li, R., Zucker, S., & Toole, B. P. (2000). EMMPRIN (CD147), an inducer of matrix metalloproteinase synthesis, also binds interstitial collagenase to the tumor cell surface. *Cancer Research*, *60*, 888–891.

Guo, W., Chen, W., Yu, W., Huang, W., & Deng, W. (2013). Small interfering RNA-based molecular therapy of cancers. *Chinese Journal of Cancer*, *32*, 488–493.

Gurunathan, S., Klinman, D. M., & Seder, R. A. (2000). DNA vaccines: Immunology, application, and optimization. *Annual Review of Immunology*, *18*, 927–974.

Guzinska-Ustimowicz, K., Pryczynicz, A., Kemona, A., & Czyzewska, J. (2009). Correlation between proliferation markers: PCNA, Ki-67, MCM-2 and antiapoptotic protein Bcl-2 in colorectal cancer. *Anticancer Research*, *29*, 3049–3052.

Haase, A. D., Jaskiewicz, L., Zhang, H., Lainé, S., Sack, R., Gatignol, A., & Filipowicz, W. (2005). TRBP, a regulator of cellular PKR and HIV-1 virus expression, interacts with Dicer and functions in RNA silencing. *EMBO Reports*, *6*, 961–967.

Häcker, G. (2000). The morphology of apoptosis. *Cell and Tissue Research*, *301*, 5–17.

Haimes, J., & Kelley, M. (2010). Demonstration of a ΔΔCq Calculation Method to Compute Relative Gene Expression from qPCR Data. USA: Thermo Scientific.

Hamilton, A. J., & Baulcombe, D. C. (1999). A Species of Small Antisense RNA in Posttranscriptional Gene Silencing in Plants. *Science*, *286*, 950–952.

Han, J., Lee, Y., Yeom, K. H., Kim, Y. K., Jin, H., & Kim, V. N. (2004). The Drosha-DGCR8 complex in primary microRNA processing. *Genes & Development*, *18*, 3016–3027.

Han, J., Lee, Y., Yeom, K. H., Nam, J. W., Heo, I., Rhee, J. K., Sohn, S. Y., Cho, Y., Zhang, B. T., & Kim, V. N. (2006). Molecular basis for the recognition of primary microRNAs by the Drosha-DGCR8 complex. *Cell*, *125*, 887–901.

Hanahan, D., & Weinberg, R. a. (2011). Hallmarks of cancer: the next generation. *Cell*, *144*, 646–74. Elsevier Inc.

Harmey, J. H., & Bouchier-Hayes, D. (2002). Vascular endothelial growth factor (VEGF), a survival factor for tumour cells: implications for anti-angiogenic therapy. *BioEssays*, *24*, 280–283.

Harmey, J. H., Bucana, C. D., Lu, W., Byrne, A. M., McDonnell, S., Lynch, C., Bouchier-Hayes, D., & Dong, Z. (2002). Lipopolysaccharide induced metastatic growth is associated with increased angiogenesis, vascular permeability and tumor cell invasion. *International Journal of Cancer*, *101*, 415–422.

Hartley, J. L., Temple, G. F., & Brasch, M. A. (2000). DNA Cloning Using In Vitro Site-Specific Recombination. *Genome Research*, *10*, 1788–1795.

Herszényi, L., Hritz, I., Lakatos, G., Varga, M. Z., & Tulassay, Z. (2012). The behavior of matrix metalloproteinases and their inhibitors in colorectal cancer. *International Journal of Molecular Sciences*, *13*, 13240–13263.

Hlatky, L. (2002). Clinical application of antiangiogenic therapy: microvessel density, what it does and doesn't tell us. *Journal of the National Cancer Institute*, *94*, 883–93.

Huang, C., Li, M., Chen, C., & Yao, Q. (2008). Small interfering RNA therapy in cancer: mechanism, potential targets, and clinical applications. *Expert Opinion on Therapeutic Targets*, *12*, 637–645.

Huang, Y. H., Bao, Y., Peng, W., Goldberg, M., Love, K., Bumcrot, D. A., Cole, G., Langer, R., Anderson, D. G., & Sawicki, J. A. (2009). Claudin-3 gene silencing with siRNA suppresses ovarian tumor growth and metastasis. *Proceedings of the National Academy of Sciences of the United States of America*, *106*, 3426–3430.

IARC. (2014a). *World cancer factsheet* (Vol. 2012). London: International Agency for Research on Cancer and Cancer Research © Copyright Cancer Research UK.

IARC. (2014b). *GLOBOCAN 2012: Cancer Incidence and Mortality Worldwide*. Available from: http://globocan.iarc.fr, accessed on 27/08/14: International Agency for Research on Cancer © IARC 2014.

Ingelheim, B. (2009). *The global burden of cancer-the facts*. © Boehringer Ingelheim GmbH.

Ito, A., Tanaka, K., Honda, H., Abe, S., Yamaguchi, H., & Kobayashi, T. (2003). Complete regression of mouse mammary carcinoma with a size greater than 15

mm by frequent repeated hyperthermia using magnetite nanoparticles. *Journal of Bioscience and Bioengineering*, *96*, 364–369.

Jia, L., Wang, H., Qu, S., Miao, X., & Zhang, J. (2008). CD147 regulates vascular endothelial growth factor - A expression, tumorigenicity, and chemosensitivity to curcumin in hepatocellular carcinoma. *IUBMB Life*, *60*, 57–63.

Jia, L., Wang, S., Zhou, H., Cao, J., Hu, Y., & Zhang, J. (2006). Caveolin-1 up-regulates CD147 glycosylation and the invasive capability of murine hepatocarcinoma cell lines. *International Journal of Biochemistry and Cell Biology*, *38*, 1584–1593.

Jin, J. S., Hsieh, D. S., Lin, Y. F., Wang, J. Y., Sheu, L. F., & Lee, W. H. (2006). Increasing expression of extracellular matrix metalloprotease inducer in renal cell carcinoma: Tissue microarray analysis of immunostaining score with clinicopathological parameters. *International Journal of Urology*, *13*, 573–580.

Jonson, A. L., Rogers, L. M., Ramakrishnan, S., & Downs Jr, L. S. (2008). Gene silencing with siRNA targeting E6/E7 as a therapeutic intervention in a mouse model of cervical cancer. *Gynecologic Oncology*, *111*, 356–364. Elsevier Inc.

Judge, A. D., Robbins, M., Tavakoli, I., Levi, J., Hu, L., Fronda, A., Ambegia, E., McClintock, K., & MacLachlan, I. (2009). Technical advance Confirming the RNAi-mediated mechanism of action of siRNA-based cancer therapeutics in mice. *The Journal of Clinical Investigation*, *119*, 661–673.

Kaestner, P., Aigner, A., & Bastians, H. (2011). Therapeutic targeting of the mitotic spindle checkpoint through nanoparticle-mediated siRNA delivery inhibits tumor growth in vivo. *Cancer Letters*, *304*, 128–136. Elsevier Ireland Ltd.

Kanekura, T., Chen, X., & Kanzaki, T. (2002). Basigin (CD147) is expressed on melanoma cells and induces tumor cell invasion by stimulating production of matrix metalloproteinases by fibroblasts. *International Journal of Cancer*, *99*, 520–528.

Kanekura, T., Miyauchi, T., Tashiro, M., & Muramatsu, T. (1991). Basigin, a new member of the immunoglobulin superfamily: genes in different mammalian species, glycosylation changes in the molecule from adult organs and possible variation in the N-terminal sequences. *Cell Structure and Function*, *16*, 23–30.

Kang, M. J., Kim, H.P., Lee, K., Yoo, Y.D., Kwon, Y.T., Kim, K. M., Kim, T.Y., & Yi, E. C. (2013). Proteomic analysis reveals that CD147/EMMPRIN confers chemoresistance in cancer stem cell-like cells. *Proteomics*, *13*, 1714–1725.

Kasinrerk, W., Fiebiger, E., Stefanová, I., Baumruker, T., Knapp, W., & Stockinger, H. (1992). Human leukocyte activation antigen M6, a member of the Ig superfamily, is the species homologue of rat OX-47, mouse basigin, and chicken HT7 molecule. *Journal of Imunology*, *149*, 847–854.

Ke, X., Li, L., Dong, H. L., & Chen, Z. N. (2012). Acquisition of anoikis resistance through CD147 upregulation: A new mechanism underlying metastasis of hepatocellular carcinoma cells. *Oncology Letters*, *3*, 1249–1254.

Kerem, M., Bedirli, N., Gurbuz, N., Ekinci, O., Bedirli, A., Akkaya, T., Sakrak, O., & Pasaoglu, H. (2007). Effects of acute fenthion toxicity on liver and kidney function and histology in rats. *Turkish Journal of Medical Science*, *37*, 281–288.

Kim, B. M., Lee, D. S., Choi, J. H., Kim, C. Y., Son, M., Suh, Y. S., Baek, K. H., Park, K. S., Sung, Y. C., & Kim, W. B. (2003). In vivo kinetics and biodistribution of a HIV-1 DNA vaccine after administration in mice. *Archives of Pharmacal Research*, *26*, 493–498.

Kim, M. H., Yuan, X., Okumura, S., & Ishikawa, F. (2002). Successful inactivation of endogenous Oct-3/4 and c-mos genes in mouse preimplantation embryos and oocytes using short interfering RNAs. *Biochemical and Biophysical Research Communications*, *296*, 1372–1377.

Kimos, M. C., Wang, S., Borkowski, A., Yang, G. Y., Yang, C. S., Perry, K., Olaru, A., Deacu, E., Sterian, A., Cottrell, J., Papadimitriou, J., Sisodia, L., Selaru, F. M., Mori, Y., Xu, Y., Yin, J., Abraham, J. M., & Meltzer, S. J. (2004). Esophagin and proliferating cell nuclear antigen (PCNA) are biomarkers of human esophageal neoplastic progression. *International Journal of Cancer*, *111*, 415–417.

Kuang, Y. H., Chen, X., Su, J., Wu, L. S., Liao, L. Q., Li, D., Chen, Z. S., & Kanekura, T. (2009). RNA interference targeting the CD147 induces apoptosis of multi-drug resistant cancer cells related to XIAP depletion. *Cancer Letters*, *276*, 189–195.

Kumar, P. U., Kumar, B. D., Annapurna, V. V, Krishna, T. P., Kalyanasundaram, S., Suresh, P., Harishankar, N., Jagadeesan, V., Hariharan, S., Naidu, A. N., Krishnaswamy, K., Rangarajan, P. N., Srinivasan, V. A., Reddy, G. S., & Sesikeran, B. (2006). Nonclinical toxicology study of recombinant-plasmid DNA anti-rabies vaccines. *Vaccine*, *24*, 2790–2798.

Landesman-Milo, D., Goldsmith, M., Ben-Arye, S. L., Witenberg, B., Brown, E., Leibovitch, S., Azriel, S., Tabak, S., Morad, V., & Peer, D. (2013). Hyaluronan grafted lipid-based nanoparticles as RNAi carriers for cancer cells. *Cancer Letters*, *334*, 221–227. Elsevier Ireland Ltd.

Ledwith, B. J., Manam, S., Troilo, P. J., Barnum, A. B., Pauley, C. J., Griffiths Ii, T. G., Harper, L. B., Beare, C. M., Bagdon, W. J., & Nichols, W. W. (2000). Plasmid DNA vaccines: Investigation of integration into host cellular DNA following intramuscular injection in mice. *Intervirology*, *43*, 258–272.

Lee, A. S. (2001). The glucose-regulated proteins: stress induction and clinical applications. *Trends in Biochemical Sciences*, *26*, 504–510.

Li, X., Jin, N., Mi, Z., Lian, H., Sun, L., Li, X., & Zheng, H. (2006). Antitumor effects of a recombinant fowlpox virus expressing Apoptin in vivo and in vitro. *International Journal of Cancer*, *119*, 2948–2957.

Lian, H., Jin, N., Li, X., Mi, Z., Zhang, J., Sun, L., Li, X., Zheng, H., & Li, P. (2007). Induction of an effective anti-tumor immune response and tumor regression by combined administration of IL-18 and Apoptin. *Cancer Imunology Immunotherapy*, *56*, 181–192.

Liang, Y. X., He, H. C., Han, Z. D., Bi, X. C., Dai, Q. S., Ye, Y. K., Qin, W. J., Zeng, G. H., Zhu, G., Xu, C. L., & Zhong, W. de. (2009). CD147 and VEGF expression in advanced renal cell carcinoma and their prognostic value. *Cancer Investigation*, *27*, 788–793.

Lindahl, K., Kindmark, A., Laxman, N., Åström, E., Rubin, C. J., & Ljunggren, Ö. (2013). Allele dependent silencing of collagen type I using small interfering RNAs targeting 3'UTR Indels - a novel therapeutic approach in osteogenesis imperfecta. *International Journal of Medical Sciences*, *10*, 1333–1343.

Liu, C., Fan, M., Xu, Q., & Li, Y. (2008). Biodistribution and expression of targeted fusion anti-caries DNA vaccine pGJA-P/VAX in mice. *Journal of Gene Medicine*, *10*, 298–305.

Livak, K. J., & Schmittgen, T. homas D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2-ΔΔCT Method. *Methods*, *25*, 402–408.

Los, M., Panigrahi, S., Rashedi, I., Mandal, S., Stetefeld, J., Essmann, F., & Schulze-Osthoff, K. (2009). Apoptin, a tumor-selective killer. *Biochimica et Biophysica Acta*, *1793*, 1335–1342. Elsevier B.V.

Luo, J., Solimini, N. L., & Elledge, S. J. (2009). Principles of Cancer Therapy: Oncogene and Non-oncogene Addiction. *Cell*, *136*, 823–837.

Maddika, S., Mendoza, F. J., Hauff, K., Zamzow, C. R., Paranjothy, T., & Los, M. (2006). Cancer-selective therapy of the future: apoptin and its mechanism of action. *Cancer Biology & Therapy*, *5*, e1–e10.

Maeda, H., Wu, J., Sawa, T., Matsumura, Y., & Hori, K. (2000). Tumor vascular permeability and the EPR effect in macromolecular therapeutics: a review. *Journal of Controlled Release*, *65*, 271–284.

Manam, S., Ledwith, B. J., Barnum, A. B., Troilo, P. J., Pauley, C. J., Harper, L. B., Griffiths II, T. G., Niu, Z., Denisova, L., Follmer, T. T., Pacchione, S. J., Wang, Z., Beare, C. M., Bagdon, W. J., & Nichols, W. W. (2001). Plasmid DNA vaccines: tissue distribution and effects of DNA sequence, adjuvants and delivery method on integration into host DNA. *Intervirology*, *43*, 273–281.

Marine, S., Bahl, A., Ferrer, M., & Buehler, E. (2012). Common seed analysis to identify off-target effects in siRNA screens. *Journal of Biomolecular Screening*, *17*, 370–378.

Martin, T., Parker, S. E., Hedstrom, R., Le, T., Hoffman, S. L., Norman, J., Hobart, P., & Lew, D. (1999). Plasmid DNA malaria vaccine: The potential for genomic integration after intramuscular injection. *Human Gene Therapy*, *10*, 759–768.

Maskos, K., & Bode, W. (2003). Structural Basis of Matrix Metalloproteinases and Tissue Inhibitors of Metalloproteinases. *Molecular Biotechnology*, *25*, 241–266.

Matranga, C., Tomari, Y., Shin, C., Bartel, D. P., & Zamore, P. D. (2005). Passenger-strand cleavage facilitates assembly of siRNA into Ago2-containing RNAi enzyme complexes. *Cell*, *123*, 607–620.

Meister, G., Landthaler, M., Peters, L., Chen, P. Y., Urlaub, H., & Luhrmann, Reinhard Tuschl, T. (2005). Identification of novel argonaute-associated proteins. *Current Biology*, *15*, 2149–2155.

Melchior, A., Denys, A., Deligny, A., Mazurier, J., & Allain, F. (2008). Cyclophilin B induces integrin-mediated cell adhesion by a mechanism involving CD98-dependent activation of protein kinase C-δ and p44/42 mitogen-activated protein kinases. *Experimental Cell Research*, *314*, 616–628.

Meyer, M., & Wagner, E. (2006). Recent developments in the application of plasmid DNA-based vectors and small interfering RNA therapeutics for cancer. *Human Gene Therapy*, *17*, 1062–1076.

Mi, L. (2006). Molecular cloning of protein-based polymers. *Biomacromolecules*, *7*, 2099–2107.

MoH. (2013). *Health Facts 2013*. Ministry of Health, Malaysia.

Mohamed, G. (2001). *The expression of chicken anemia virus VP3 gene and induction of apoptosis in transformed and tumor cells*. Universiti Putra Malaysia.

Muramatsu, T., & Miyauchi, T. (2003). Basigin (CD147): a multifunctional transmembrane protein involved in reproduction, neural function, inflammation and tumor invasion. *Histology and Histopathology*, *18*, 981–987.

Nabeshima, K., Iwasaki, H., Koga, K., Hojo, H., Suzumiya, J., & Kikuchi, M. (2006). Emmprin (basigin/CD147): matrix metalloproteinase modulator and multifunctional cell recognition molecule that plays a critical role in cancer progression. *Pathology International*, *56*, 359–367.

Napoli, C., Lemieux, C., & Jorgensen, R. (1990). Introduction of a Chimeric Chalcone Synthase Gene into Petunia Results in Reversible Co-Suppression of Homologous Genes in trans. *The Plant Cell*, *2*, 279–289.

Natesan, S., Kataria, J. M., Dhama, K., Bhardwaj, N., & Sylvester, A. (2006). Anti-neoplastic effect of chicken anemia virus VP3 protein (apoptin) in Rous sarcoma virus-induced tumours in chicken. *Journal of General Virology*, *87*, 2933–2940.

Naumova, N., Smith, E. M., Zhan, Y., & Dekker, J. (2012). Analysis of long-range chromatin interactions using Chromosome Conformation Capture. *Methods*, *58*, 192–203.

Nehme, C. L., Cesario, M. M., Myles, D. G., Koppel, D. E., & Bartles, J. R. (1993). Breaching the diffusion barrier that compartmentalizes the transmembrane glycoprotein CE9 to the posterior-tail plasma membrane domain of the rat spermatozoon. *The Journal of Cell Biology*, *120*, 687–694.

Ni, M., & Lee, A. S. (2007). ER chaperones in mammalian development and human diseases. *FEBS Letters*, *581*, 3641–51.

Nik Abd Rahman, N. M. A., Allaudin, Z. N., Mohd M, N., Ismail, R., Mustafa, N., & Mohd Lila, M. A. (2011). Apoptosis and tumour cell death in response to pro-apoptotic gene. *Pertanika Journal of Tropical Agriculture Science*, *34*, 163–166.

Nishibaba, R., Higashi, Y., Su, J., Furukawa, T., Kawai, K., & Kanekura, T. (2012). CD147-targeting siRNA inhibits cell-matrix adhesion of human malignant melanoma cells by phosphorylating focal adhesion kinase. *The Journal of Dermatology*, *39*, 63–67.

Noguchi, Y., Wu, J., Duncan, R., Strohalm, J., Ulbrich, K., Akaike, T., & Maeda, H. (1998). Early phase tumor accumulation of Macromolecules: A great difference in clearance rate between tumor and normal tissues. *Japanese Journal of Cancer Research*, *89*, 307–314.

Noteborn, M. H. M., Todd, D., Verschueren, C. A. J., De Gauw, H. W. F. M., Curran, W. L., Veldkamp, S., Douglas, A. J., McNulty, M. S., Van der Eb, A. J., & Koch, G. (1994). A single chicken anemia virus protein induces apoptosis. *Journal of Virology*, *68*, 346–351.

Ochrietor, J. D., Moroz, T. M., Kadomatsu, K., Muramatsu, T., & Linser, P. J. (2001). Retinal degeneration following failed photoreceptor maturation in 5A11/basigin null mice. *Experimental Eye Research*, *72*, 467–477.

Ogris, M., & Wagner, E. (2002). Targeting tumors with non-viral gene delivery systems. *Drug Discovery Today*, *7*, 479–485.

Omar, Z. A., & Ibrahim Tamin, N. S. (2011). *National Cancer Registry Report Malaysia Cancer Statistics – Data and Figure 2007*. National Cancer Registry Ministry of Health, Malaysia.

Orság, P., Kvardová, V., Raška, M., Miller, A. D., Ledvina, M., & Turánek, J. (2008). Quantitative real-time PCR study on persistence of pDNA vaccine pVax-Hsp60 TM814 in beef muscles. *Genetic Vaccines and Therapy*, *6*, 11.

Osaka, G., Carey, K., Cuthbertson, A., Godowski, P., Patapoff, T., Ryan, A., Gadek, T., & Mordenti, J. (1996). Pharmacokinetics, tissue distribution, and expression Efficiency of plasmid [33P]DNA following intravenous administration of DNA/cationic lipid complexes in mice: Use of a novel radionuclide approach. *Journal of Pharmaceutical Sciences*, *85*, 612–618.

Paoli, P., Giannoni, E., & Chiarugi, P. (2013). Anoikis molecular pathways and its role in cancer progression. *Biochimica et Biophysica Acta*, *1833*, 3481–3498. Elsevier B.V.

Parker, R., Phan, T., Baumeister, P., Roy, B., Cheriyath, V., Roy, A. L., & Lee, A. S. (2001). Identification of TFII-I as the Endoplasmic Reticulum Stress Response Element Binding Factor ERSF: Its Autoregulation by Stress and Interaction with ATF6. *Molecular and Cellular Biology*, *21*, 3220–3233.

Parrish, S., Fleenor, J., Xu, S., Mello, C., & Fire, A. (2000). Functional Anatomy of a dsRNA Trigger: Differential Requirement for the Two Trigger Strands in RNA Interference. *Molecular Cell*, *6*, 1077–1087.

Pastorino, F., Brignole, C., Di Paolo, D., Nico, B., Pezzolo, A., Marimpietri, D., Pagnan, G., Piccardi, F., Cilli, M., Longhi, R., Ribatti, D., Corti, A., Allen, T. M., & Ponzoni, M. (2006). Targeting liposomal chemotherapy via both tumor cell-specific and tumor vasculature-specific ligands potentiates therapeutic efficacy. *Cancer Research*, *66*, 10073–82.

Peng, D. J., Sun, J., Wang, Y. Z., Tian, J., Zhang, Y. H., Noteborn, M. H. M., & Qu, S. (2007). Inhibition of hepatocarcinoma by systemic delivery of Apoptin gene via the hepatic asialoglycoprotein receptor. *Cancer Gene Therapy*, *14*, 66–73.

Persano, L., Crescenzi, M., & Indraccolo, S. (2007). Anti-angiogenic gene therapy of cancer: current status and future prospects. *Molecular Aspects of Medicine*, *28*, 87–114.

Pidgeon, G., Harmey, J., Kay, E., Da Costa, M., Redmond, H., & Bouchier-Hayes, D. (1999). The role of endotoxin/lipopolysaccharide in surgically induced tumour growth in a murine model of metastatic disease. *British Journal of Cancer*, *81*, 1311–7.

Pietersen, A. M., Van Der Eb, M. M., Rademaker, H. J., Van Den Wollenberg, D. J. M., Rabelink, M. J. W. E., Kuppen, P. J. K., Van Dierendonck, J. H., Van Ormondt, H., Masman, D., Van De Velde, C. J. H., Van Der Eb, A. J., Hoeben, R. C., & Noteborn, M. H. M. (1999). Specific tumor-cell killing with adenovirus vectors containing the apoptin gene. *Gene Therapy*, *6*, 882–892.

Pozzi, A., & Capdevila, J. H. (2008). PPARalpha Ligands as Antitumorigenic and Antiangiogenic Agents. *PPAR Research*, 906542.

Prochazkova, J., & Kylarova, D. (2003). Comparative study of apoptosis-detecting techniques: TUNEL, apostain, and lamin B. *BioTechniques*, *35*, 528–534.

Pytliak, M., Vargová, V., & Mechírová, V. (2012). Matrix metalloproteinases and their role in oncogenesis: a review. *Onkologie*, *35*, 49–53.

Qasim, B. J., Hussein, A. G., & Ali, H. H. (2012). Immunohistochemical expression of PCNA and CD34 in colorectal adenomas and carcinomas using specified automated cellular image analysis system: A clinicopathologic study. *Saudi Journal of Gastroenterology*, *18*, 268–276.

Qin, L., Ding, Y., Pahud, D. R., Chang, E., Imperiale, M. J., & Bromberg, J. S. (1997). Promoter Attenuation in Gene Therapy: Interferon-y and Tumor Necrosis Factor-a Inhibit Transgene Expression. *Human Gene Therapy*, *8*, 2019–2029. Mary Ann Liebert, Inc., publishers.

Querbes, W., Ge, P., Zhang, W., Fan, Y., Costigan, J., Charisse, K., Maier, M., Nechev, L., Manoharan, M., Kotelianski, V., & Sah, D. W. Y. (2009). Direct CNS delivery of siRNA mediates robust silencing in oligodendrocytes. *Oligonucleotides*, *19*, 23–29.

Rainaldi, G., Romano, R., Indovina, P., Ferrante, A., Motta, A., Indovina, P. L., & Santini, M. T. (2008). Metabolomics using 1H-NMR of apoptosis and Necrosis in HL60 leukemia cells: differences between the two types of cell death and independence from the stimulus of apoptosis used. *Radiation Research*, *169*, 170–180.

Rao, D. D., Vorhies, J. S., Senzer, N., & Nemunaitis, J. (2009). siRNA vs. shRNA: similarities and differences. *Advanced Drug Delivery Reviews*, *61*, 746–759. Elsevier B.V.

Rettig, G. R., & Behlke, M. A. (2012). Progress toward in vivo use of siRNAs-II. *Molecular Therapy*, *20*, 483–512. Nature Publishing Group.

Reynolds, A., Leake, D., Boese, Q., Scaringe, S., Marshall, W. S., & Khvorova, A. (2004). Rational siRNA design for RNA interference. *Nature Biotechnology*, *22*, 326–330.

Reynolds, C. P., Sun, B. C., DeClerck, Y. A., & Moats, R. A. (2005). Assessing growth and response to therapy in murine tumor models. *Methods in Molecular Medicine*, *111*, 335–350.

Rivas, F. V, Tolia, N. H., Song, J. J., Aragon, J. P., Liu, J., Hannon, G. J., & Joshua-Tor, L. (2005). Purified Argonaute2 and an siRNA form recombinant human RISC. *Nature Structural & Molecular Biology*, *12*, 340–349.

Rmali, K. A., Puntis, M. C. A., & Jiang, W. G. (2006). Tumour-associated angiogenesis in human colorectal cancer. *Colorectal Disease*, *9*, 3–14.

Roskoski, R. (2007). Vascular endothelial growth factor (VEGF) signaling in tumor progression. *Critical Reviews in Oncology/Hematology*, *62*, 179–213.

Sancéau, J., Poupon, M.F., Delattre, O., Sastre-Garau, X., & Wietzerbin, J. (2002). Strong inhibition of Ewing tumor xenograft growth by combination of human interferon-alpha or interferon-beta with ifosfamide. *Oncogene*, *21*, 7700–7709.

Schellerer, V. S., Mueller-Bergh, L., Merkel, S., Zimmermann, R., Weiss, D. R., Schlabrakowski, A., Naschberger, E., Stürzl, M., Hohenberger, W., & Croner, R. S. (2011). The clinical value of von Willebrand factor in colorectal carcinomas. *American Journal of Translational Research*, *3*, 445–453.

Scheule, R. K. (2000). The role of CpG motifs in immunostimulation and gene therapy. *Advanced Drug Delivery Reviews*, *44*, 119–134.

Schneiderhan, W., Scheler, M., Holzmann, K.-H., Marx, M., Gschwend, J. E., Bucholz, M., Gress, T. M., Seufferlein, T., Adler, G., & Oswald, F. (2009). CD147 silencing inhibits lactate transport and reduces malignant potential of pancreatic cancer cells in in vivo and in vitro models. *Gut*, *58*, 1391–1398.

Schoop, R. A. L., Baatenburg de Jong, R. J., & Noteborn, M. H. M. (2008). Apoptin induces apoptosis in an oral cancer mouse model. *Cancer Biology & Therapy*, *7*, 1368–1373.

Schoop, R. A. L., Kooistra, K., Baatenburg De Jong, R. J., & Noteborn, M. H. M. (2004). Bcl-xL inhibits p53- but not apoptin-induced apoptosis in head and neck squamous cell carcinoma cell line. *International Journal of Cancer*, *109*, 38–42.

Sen, G. L., & Blau, H. M. (2005). Argonaute 2/RISC resides in sites of mammalian mRNA decay known as cytoplasmic bodies. *Nature Cell Biology*, *7*, 633–636.

Sengupta, N., & MacDonald, T. T. (2007). The role of matrix metalloproteinases in stromal/epithelial interactions in the gut. *Physiology*, *22*, 401–409.

Seth, S., Matsui, Y., Fosnaugh, K., Liu, Y., Vaish, N., Adami, R., Harvie, P., Johns, R., Severson, G., Brown, T., Takagi, A., Bell, S., Chen, Y., Chen, F., Zhu, T., Fam, R., Maciagiewicz, I., Kwang, E., McCutcheon, M., Farber, K., Charmley, P., Houston, M. E., So, A., Templin, M. V, & Polisky, B. (2011). RNAi-based therapeutics targeting survivin and PLK1 for treatment of bladder cancer. *Molecular Therapy*, *19*, 928–935. Nature Publishing Group.

Seulberger, H., Lottspeich, F., & Risau, W. (1990). The inducible blood--brain barrier specific molecule HT7 is a novel immunoglobulin-like cell surface glycoprotein. *The EMBO Journal*, *9*, 2151–2158.

Seulberger, H., Unger, C. M., & Risau, W. (1992). HT7, Neurothelin, Basigin, gp42 and OX-47 - Many names for one developmentally regulated immuno-globulin-like surface glycoprotein on blood-brain barrier endothelium, epithelial tissue barriers and neurons. *Neuroscience Letters*, *140*, 93–97.

Shao, Y., Chan, C. Y., Maliyekkel, A., Lawrence, C. E., Roninson, I. B., & Ding, Y. (2007). Effect of target secondary structure on RNAi efficiency. *RNA*, *13*, 1631–1640.

Sharpless, N. E., & Depinho, R. A. (2006). The mighty mouse: genetically engineered mouse models in cancer drug development. *Nature Reviews Drug Discovery*, *5*, 741–754.

Singhal, S. S., Awasthi, Y. C., & Awasthi, S. (2006). Regression of melanoma in a murine model by RLIP76 depletion. *Cancer Research*, *66*, 2354–2360.

Sinn, P., Sauter, S., & McCray Jr, P. (2005). Gene therapy progress and prospects: development of improved lentiviral and retroviral vectors-design, biosafety, and production. *Gene Therapy*, *12*, 1089–1098.

Snoek-van Beurden, P. A. M., & Von den Hoff, J. W. (2005). Zymographic techniques for the analysis of matrix metalloproteinases and their inhibitors. *BioTechniques*, *38*, 73–83.

Soutschek, J., Akinc, A., Bramlage, B., Charisse, K., Constien, R., Donoghue, M., Elbashir, S., Geick, A., Hadwiger, P., Harborth, J., John, M., Kesavan, V., Lavine, G., Pandey, R. K., Racie, T., Rajeev, K. G., Röhl, I., Toudjarska, I., Wang, G., Wuschko, S., Bumcrot, D., Koteliansky, V., Limmer, S., Manoharan, M., & Vornlocher, H.-P. (2004). Therapeutic silencing of an endogenous gene by systemic administration of modified siRNAs. *Nature*, *432*, 173–178.

Starke, R. D., Ferraro, F., Paschalaki, K. E., Dryden, N. H., McKinnon, T. A. J., Sutton, R. E., Payne, E. M., Haskard, D. O., Hughes, A. D., Cutler, D. F., Laffan, M. a, & Randi, A. M. (2011). Endothelial von Willebrand factor regulates angiogenesis. *Blood*, *117*, 1071–1080.

Stenzinger, A., Wittschieber, D., von Winterfeld, M., Goeppert, B., Kamphues, C., Weichert, W., Dietel, M., Rabien, A., & Klauschen, F. (2012). High extracellular matrix metalloproteinase inducer/CD147 expression is strongly and independently associated with poor prognosis in colorectal cancer. *Human Pathology*, *43*, 1471–1481. Elsevier Inc.

Stewart, I., Schluter, P. J., & Shaw, G. R. (2006). Cyanobacterial lipopolysaccharides and human health - a review. *Environmental Health: A Global Access Science Source*, *5*, 7.

Su, J., Chen, X., & Kanekura, T. (2009). A CD147-targeting siRNA inhibits the proliferation, invasiveness, and VEGF production of human malignant melanoma cells by down-regulating glycolysis. *Cancer Letters*, *273*, 140–147.

Sun, J., & Hemler, M. E. (2001). Regulation of MMP-1 and MMP-2 production through CD147/extracellular matrix metalloproteinase inducer interactions. *Cancer Research*, *61*, 2276–2281.

Suo, W. H., Zhang, N., Wu, P. P., Zhao, L., Song, L. J., Shen, W. W., Zheng, L., Tao, J., Long, X. D., & Fu, G. H. (2012). Anti-tumour effects of small interfering RNA targeting anion exchanger 1 in experimental gastric cancer. *British Journal of Pharmacology*, *165*, 135–147.

Suria, M. S. (2006). *Development of a recombinant retrovirus expressing the chicken anemia virus Vp3 protein*. Universiti Putra Malaysia.

Talmadge, J. E., Singh, R. K., Fidler, I. J., & Raz, A. (2007). Murine models to evaluate novel and conventional therapeutic strategies for cancer. *The American Journal of Pathology*, *170*, 793–804.

Tam, P., Monck, M., Lee, D., Ludkovski, O., Leng, E., Clow, K., Stark, H., Scherrer, P., Graham, R., & Cullis, P. R. (2000). Stabilized plasmid-lipid particles for systemic gene therapy. *Gene Therapy*, *7*, 1867–1874.

Tammali, R., B.M.Reddy, A., Saxena, A., G.Rychahou, P., Evers, B. M., Qiu, S., Awasthi, S., V.Ramana, K., & K.Srivastava, S. (2011). Inhibition of aldose reductase prevents colon cancer metastasis. *Carcinogenesis*, *32*, 1259–1267.

Tang, J., Zhou, H., Jiang, J., Yang, X., Li, Y., Zhang, H., Chen, Z., & Guo, W. (2007). BetaIg-h3 is involved in the HAb18G/CD147-mediated metastasis process in human hepatoma cells. *Experimental Biology and Medicine*, *232*, 344–352.

Tang, W., & Hemler, M. E. (2004). Caveolin-1 regulates matrix metalloproteinases-1 induction and CD147/EMMPRIN cell surface clustering. *The Journal of Biological Chemistry*, *279*, 11112–11118.

Tang, Y., Nakada, M. T., Kesavan, P., McCabe, F., Millar, H., Rafferty, P., Bugelski, P., & Yan, L. (2005). Extracellular Matrix Metalloproteinase Inducer Stimulates Tumor Angiogenesis by Elevating Vascular Endothelial Cell Growth Factor and Matrix Metalloproteinases Growth Factor and Matrix Metalloproteinases. *Cancer Research*, *65*, 3193–3199.

Taylor, P. M., Woodfield, R. J., Hodgkin, M. N., Pettitt, T. R., Martin, A., Kerr, D. J., & Wakelam, M. J. O. (2002). Breast cancer cell-derived EMMPRIN stimulates fibroblast MMP2 release through a phospholipase A(2) and 5-lipoxygenase catalyzed pathway. *Oncogene*, *21*, 5765–5772.

Teicher, B. A. (2011). Preclinical tumor response end points. In B. A. Teicher (Ed.), *Tumor Models in Cancer Research* (pp. 571–605). Totowa, NJ: Humana Press.

Tesniere, A., Abermil, N., Schlemmer, F., Casares, N., Kepp, O., Pequignot, M., Michaud, M., Martins, I., Senovilla, L., Zitvogel, L., & Kroemer, G. (2010). In vivo depletion of T lymphocyte-specific transcription factors by RNA interference. *Cell Cycle*, *9*, 2902–2907.

Toth, M., & Fridman, R. (2001). Assesment of Gelatinases (MMP-2 and MMP-9) by Gelatin Zymography. *Methods in Molecular Medicine*, *57*, 163–174.

Tuomela, M., Malm, M., Wallen, M., Stanescu, I., Krohn, K., & Peterson, P. (2005). Biodistribution and general safety of a naked DNA plasmid, GTU®-MultiHIV, in a rat, using a quantitative PCR method. *Vaccine*, *23*, 890–896.

Vaishnaw, A. K., Gollob, J., Gamba-vitalo, C., Hutabarat, R., Sah, D., Meyers, R., De, T., & Maraganore, J. (2010). A status report on RNAi therapeutics. *Silence*, *1*, 1–13.

Verma, I. M., & Weitzman, M. D. (2005). Gene therapy: twenty-first century medicine. *Annual Review of Biochemistry*, *74*, 711–738.

Vermes, I., Haanen, C., & Reutelingsperger, C. (2000). Flow cytometry of apoptotic cell death. *Journal of Immunological Methods*, *243*, 167–190.

Vilalta, A., Mahajan, R. K., Hartikka, J., Rusalov, D., Martin, T., Bozoukova, V., Leamy, V., Hall, K., Lalor, P., Rolland, A., & Kaslow, D. C. (2005). I. Poloxamer-formulated plasmid DNA-based human cytomegalovirus vaccine: Evaluation of plasmid DNA biodistribution/persistence and integration. *Human Gene Therapy*, *16*, 1143–1150.

Visser, A. E., Backendorf, C., & Noteborn, M. (2007). Viral protein apoptin as a molecular tool and therapeutic bullet: implications for cancer control. *Future Virology*, *2*, 519–527. Future Medicine.

Voigt, H., Vetter-Kauczok, C. S., Schrama, D., Hofmann, U. B., Becker, J. C., & Houben, R. (2009). CD147 impacts angiogenesis and metastasis formation. *Cancer Investigation*, *27*, 329–333.

Vulcano, M., Meiss, R. P., & Isturiz, M. A. (2000). Deferoxamine reduces tissue injury and lethality in LPS-treated mice. *International Journal of Immunopharmacology*, *22*, 635–644.

Wang, B., Xu, Y. F., He, B. S., Pan, Y. Q., Zhang, L. R., Zhu, C., Qu, L. L., & Wang, S. K. (2010). RNAi-mediated silencing of CD147 inhibits tumor cell proliferation, invasion and increases chemosensitivity to cisplatin in SGC7901 cells in vitro. *Journal of Experimental & Clinical Cancer Research*, *29*, 1–8.

Wang, L., Zhang, Z. G., Zhang, R. L., Gregg, S. R., Hozeska-Solgot, A., LeTourneau, Y., Wang, Y., & Chopp, M. (2006). Matrix metalloproteinase 2 (MMP2) and MMP9 secreted by erythropoietin-activated endothelial cells promote neural progenitor cell migration. *The Journal of Neuroscience*, *26*, 5996–6003.

Wang, Z., Troilo, P. J., Wang, X., Griffiths II, T. G., Pacchione, S. J., Barnum, A. B., Harper, L. B., Pauley, C. J., Niu, Z., Denisova, L., Follmer, T. T., Rizzuto, G., Ciliberto, G., Fattori, E., Monica, N. L., Manam, S., & Ledwith, B. J. (2004). Detection of integration of plasmid DNA into host genomic DNA following intramuscular injection and electroporation. *Gene Therapy*, *11*, 711–721.

Weidle, U. H., Scheuer, W., Eggle, D., Klostermann, S., & Stockinger, H. (2010). Cancer-related issues of CD147. *Cancer Genomics & Proteomics*, *7*(3), 157–69.

Whelan, J. A., Russell, N. B., & Whelan, M. A. (2003). A method for the absolute quantification of cDNA using real-time PCR. *Journal of Immunological Methods*, *278*, 261–269.

Wilson, M. C., Meredith, D., & Halestrap, A. P. (2002). Fluorescence resonance energy transfer studies on the interaction between the lactate transporter MCT1 and CD147 provide information on the topology and stoichiometry of the complex in situ. *The Journal of Biological Chemistry*, *277*, 3666–3672.

Won, Y. W., Yoon, S. M., Lee, K. M., & Kim, Y. H. (2011). Poly(oligo-D-arginine) with internal disulfide linkages as a cytoplasm-sensitive carrier for siRNA delivery. *Molecular Therapy*, *19*, 372–380. Nature Publishing Group.

Wong, R. S. Y. (2011). Apoptosis in cancer: from pathogenesis to treatment. *Journal of Experimental & Clinical Cancer Research*, *30*(87), 1–14. BioMed Central Ltd.

Xiao, L., Yan, L., Zhongmei, W., & Chang, L. (2010). Potent anti-tumor effects of a dual specific oncolytic adenovirus expressing apoptin in vitro and in vivo. *Molecular Cancer*, *9*, 1–12.

Xu, D., & Hemler, M. E. (2005). Metabolic activation-related CD147-CD98 complex. *Molecular & Cellular Proteomics*, *4*, 1061–1071.

Yagi, N., Manabe, I., Tottori, T., Ishihara, A., Ogata, F., Kim, J. H., Nishimura, S., Fujiu, K., Oishi, Y., Itaka, K., Kato, Y., Yamauchi, M., & Nagai, R. (2009). A nanoparticle system specifically designed to deliver short interfering RNA inhibits tumor growth in vivo. *Cancer Research*, *69*, 6531–6538.

Yang, L., & Carbone, D. P. (2004). Tumor-host immune interactions and dendritic cell dysfunction. *Advances in Cancer Research*, *92*, 13–27.

Yang, Y., Lu, N., Zhou, J., Chen, Z. N., & Zhu, P. (2008). Cyclophilin A up-regulates MMP-9 expression and adhesion of monocytes/ macrophages via CD147 signalling pathway in rheumatoid arthritis. *Rheumatology*, *47*, 1299–1310.

Ye, Q. F., Zhang, Y. C., Peng, X. Q., Long, Z., Ming, Y. Z., & He, L. Y. (2012). Silencing Notch-1 induces apoptosis and increases the chemosensitivity of prostate cancer cells to docetaxel through Bcl-2 and Bax. *Oncology Letters*, *3*, 879–884.

Yew, N. S. (2005). Controlling the kinetics of transgene expression by plasmid design. *Advanced Drug Delivery Reviews*, *57*, 769–780.

Yew, N. S., Przybylska, M., Ziegler, R. J., Liu, D., & Cheng, S. H. (2001). High and sustained transgene expression in vivo from plasmid vectors containing a hybrid ubiquitin promoter. *Molecular Therapy*, *4*, 75–82.

Yi, R., Doehle, B. P., Qin, Y., Macara, I. G., & Cullen, B. R. (2005). Overexpression of Exportin 5 enhances RNA interference mediated by short hairpin RNAs and microRNAs. *RNA*, *11*, 220–226.

Yilmaz, G., Gursoy-Ozdemir, Y., & Dogan, A. (2001). Spleen damage in endotoxaemic mice: the involvement of nitric oxide. *Journal of Physiology and Pharmacology*, *52*, 729–744.

Yoshizawa, T., Hattori, Y., Hakoshima, M., Koga, K., & Maitani, Y. (2008). Folate-linked lipid-based nanoparticles for synthetic siRNA delivery in KB tumor xenografts. *European Journal of Pharmaceutics and Biopharmaceutics*, *70*, 718–725. Elsevier B.V.

Yu, J. Y., Taylor, J., DeRuiter, S. L., Vojtek, A. B., & Turner, D. L. (2003). Simultaneous inhibition of GSK3α and GSK3β using hairpin siRNA expression vectors. *Molecular Therapy*, *7*, 228–236.

Yu, P., Huang, B., Shen, M., Lau, C., Chan, E., Michel, J., Xiong, Y., Payan, D. G., & Luo, Y. (2001). p15 PAF, a novel PCNA associated factor with increased expression in tumor tissues. *Oncogene*, *20*, 484–489.

Yuan, L., Zhao, H., Zhang, L., & Liu, X. (2013). The efficacy of combination therapy using adeno-associated virus-mediated co-expression of apoptin and interleukin-24 on hepatocellular carcinoma. *Tumor Biology*, *34*, 3027–3034.

Yurchenko, V., Constant, S., Eisenmesser, E., & Bukrinsky, M. (2010). Cyclophilin-CD147 interactions: a new target for anti-inflammatory therapeutics. *Clinical and Experimental Immunology*, *160*, 305–317.

Yurchenko, V., O'Connor, M., Dai, W. W., Guo, H., Toole, B., Sherry, B., & Bukrinsky, M. (2001). CD147 is a signaling receptor for cyclophilin B. *Biochemical and Biophysical Research Communications*, *288*, 786–788.

Yurchenko, V., Zybarth, G., O'Connor, M., Dai, W. W., Franchin, G., Hao, T., Guo, H., Hung, H.-C., Toole, B., Gallay, P., Sherry, B., & Bukrinsky, M. (2002). Active site residues of cyclophilin A are crucial for its signaling activity via CD147. *The Journal of Biological Chemistry*, *277*, 22959–22965.

Zamore, P. D., Tuschl, T., Sharp, P. A., & Bartel, D. P. (2000). RNAi: Double-Stranded RNA Directs the ATP-Dependent Cleavage of mRNA at 21 to 23 Nucleotide Intervals. *Cell*, *101*, 25–33.

Zhang, H. Y., Sun, S. H., Guo, Y. J., Chen, Z. H., Huang, L., Gao, Y. J., Wan, B., Zhu, W. J., Xu, G. X., & Wang, J. J. (2005). Tissue distribution of a plasmid DNA containing epitopes of foot-and-mouth disease virus in mice. *Vaccine*, *23*, 5632–5640.

Zhang, Q., Zhou, J., Ku, X. M., Chen, X. G., Zhang, L., Xu, J., Chen, G. S., Li, Q., Qian, F., Tian, R., Wen, N., & Chen, Z. N. (2007). Expression of CD147 as a significantly unfavorable prognostic factor in hepatocellular carcinoma. *European Journal of Cancer Prevention*, *16*, 196–202.

Zhang, Y., Kooistra, K., Pietersen, A., Rohn, J. L., & Noteborn, M. H. M. (2004). Activation of the tumor-specific death effector apoptin and its kinase by an N-terminal determinant of Simian Virus 40 large T antigen. *Journal of Virology*, *78*, 9965–9976.

Zhao, Y., Chen, S., Gou, W. fng, Niu, Z., Zhao, S., Xiao, L., Takano, Y., & Zheng, H. (2013). The role of EMMPRIN expression in ovarian epithelial carcinomas. *Cell Cycle*, *12*, 2899–2913.

Zheng, H. C., Takahashi, H., Murai, Y., Cui, Z. G., Nomoto, K., Miwa, S., Tsuneyama, K., & Takano, Y. (2006). Upregulated EMMPRIN/CD147 might contribute to growth and angiogenesis of gastric carcinoma: a good marker for local invasion and prognosis. *British Journal of Cancer*, *95*, 1371–1378.

Zhou, Q. H., Wu, C., Manickam, D. S., & Oupický, D. (2009). Evaluation of pharmacokinetics of bioreducible gene delivery vectors by real-time PCR. *Pharmaceutical Research*, *26*, 1581–1589.

Zhu, X., Song, Z., Zhang, S., Nanda, A., & Li, G. (2014). CD147: a Novel Modulator of Inflammatory and Immune Disorders. *Current Medicinal Chemistry*, *21*, 2138–2145.

Ziegler, U., & Groscurth, P. (2004). Morphological Features of Cell Death. *News in Physiological Sciences*, *19*, 124–128.

Zou, W., Yang, H., Hou, X., Zhang, W., Chen, B., & Xin, X. (2007). Inhibition of CD147 gene expression via RNA interference reduces tumor cell invasion, tumorigenicity and increases chemosensitivity to paclitaxel in HO-8910pm cells. *Cancer Letters*, *248*, 211–218.