ANTI-CANCER STUDIES OF *GARCINIA MANGOSTANA* L. AND *SYZYGIUM CAMPANULATUM* KORTH TOWARDS COLORECTAL CARCINOMA

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UNIVERSITI SAINS MALAYSIA

2011

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by

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Thesis submitted in fulfillment of the requirements for the degree of Doctor of Philosophy

December 2011

This thesis is dedicated to …

my mother

brothers and sisters

and to

my beloved wife and daughters

ACKNOWLEDGMENT

First and foremost praise to Allah the almighty who gave me the knowledge, strength and the determination to finish my thesis successfully.

I offer my sincerest gratitude to my supervisor, Dr Amin Malik Shah Abdul Majid, who has supported me throughout my study with his patience and knowledge whilst allowing me the room to work in my own way. I attribute the level of my PhD degree to his encouragement and effort and without him this thesis would not have been completed.

Deepest gratitude are also due to my co-supervisors, Professor Khalid M. Abu-Salah and Professor Zhari Ismail, without their invaluable assistance, support and guidance, this study would not have been completed.

I would like to thank Universiti Sains Malaysia for giving me the opportunity and providing me with all the necessary facilities that made my study possible. I would like also to thank USM for awarding me the postgraduate fellowship for two years, which has supported me during my study. I am also grateful to the School of Pharmaceutical Sciences for giving me the opportunity to carry out my study, and to all the laboratory staff who helped me in any respect during the completion of the project.

This project received funds from the Malaysian Ministry of Science and Technology (MOSTI) science fund and by the Malaysian Ministry of Higher Education. The project was also partially funded by the research chair of cancer targeting and treatment using nanoparticles, King Saud University.

This project would not have been possible without the kind cooperation and help of several people and departments. Dr. Mohammad Jamshed Siddiqui helped in the

phytochemistry work, Dr. Khadeer Ahmad has helped in the collection of the blood samples in the acute oral toxicity study, Dr. Gurjeet Kaur helped in the histopathology study of the acute oral toxicity and the anti-tumor studies, Dr. Tan Mei Lan provided the fluorescent microscope, Professor Sharif Mahsufi Mansor and Associate Professor Sabariah Ismail provided the luminescent microplate reader. Miss. Norshirin Idris and Miss. Syima Muslim have helped in purchasing the consumables and in following up in the administration matters, Mr. Zeyad Nassar has participated in implanting new techniques which have been used in this study, Mr. Shanmugan A/C Vellosamy authenticated the studied plants, Mr. Mohammad Razak Hamdan helped in the LC-MS spectrometry. Mr. Juzaili Azizi and Miss. Nur Aziah Hanapi helped in the luminescence measurements, Mr. Ahmad Ismail helped in the fluorescent microscopy studies, and Miss. Siti Marina Maidin translated the thesis abstract into the Malay language. Departments of Pharmacology, Pharmaceutical Chemistry, the drug center, the Centre for Herbal Standardization (CHEST) provided the equipments used in this study and Evonik Degussa Corporation provided the Eudragit polymers.

Special thanks to my colleagues at EMAN research laboratory. I am also thankful to all of my friends for their encouragement and support.

Last but not least, I wish to express my deepest love and gratitude to my beloved wife Sukayna and my daughters Marah and Jana; for their support, understanding and endless love, through the duration of my studies.

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CHAPTER ONE – LIRATURE REVIEW

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LIST OF ABBREVIATIONS

ADME	Absorption, Distribution, Metabolism, and Excretion
18q21	Chromosome 18, long arm band 2, sub-band 1
5-FU	5-Fluorouracil
AIDS	Acquired Immuno-Deficiency Syndrome
Akt	AKT8 virus oncogene cellular homolog
ALT	Alanine Aminotranferease
ANAOVA	Analysis of Variance
Ang-1 and 2	Angiopoietins 1 and 2
AP-1	Activator protein-1
APAF-1	Apoptotic Protease Activating Factor-1
APC	Adenomatous Polypsis Coli
AST	Aspartate Aminotransferase
ATCC	American Type Culture Collection
ATR/FTIR	Attenuated Total Reflection Fourier Transform Infrared
BA	Betulinic Acid
Bad	Bcl-xL/Bcl_2-associated death promoter
Bax	Bcl_2 associated X protein
Bcl_2	B-cell lymphoma 2
Bcl-xL	B-cell lymphoma extra large
bFGF	basic Fibroblast Growth Factor
bp	base pair

BSA	Bovine Serum Albumin
CAM	chorioallantoic membrane
Cap	Capecitabine
CapOx	Capecitabine/oxaliplatin
CCD-18Co	Normal human fibroblast
c-Fos	Member of AP-1 family of transcription factors
c-Jun	Member of AP-1 family of transcription factors
CMC	Critical Micellar Concentration
CMV	Cytomegalovirus
c-Myc	Myelocytomatosis oncogene cellular homolog
CO_2	Carbon Dioxide
COX	Cyclooxygenases
COX- 1 and 2	Cyclooxygenase-1 and 2
CuKα	Copper-K-alpha
CV	Coefficient of Variation
DAD	Diode Array Detector
DCC	Deleted in Colorectal Carcinoma
DCM	Dichloromethane
DFF	DNA Fragmentation Factor
DLS	Dynamic Light Scattering
DMEM	Dulbecco's Modified Eagle's Medium
DMH	1,2-dimethylhydrazine
DMSO	Dimethylsulfoxide

DNA	Deoxyribonucleic Acid
DPPH	2,2-Diphenyl-1-Picrylhydrazyl
DSC	Differential Scanning Calorimetry
E2F	Transcription factor family including E2F- and DP-like subunits
ECGS	Endothelial Cell Growth Supplements
ECM	Extracellular Matrix
EDTA	Ethylenediaminetetraacetic Acid
EGF	Epidermal Growth Factor
EGFR	Epidermal Growth Factor Receptor
ELISA	Enzyme-Linked Immunosorbent Assay
EPCs	Endothelial Progenitor Cells
ERK 1, 2 and 5	Extracellular signal Regulated Kinases 1, 2 and 5
ESI	Electrospray Ionization
Fas	Tumor necrosis factor superfamily receptor 6
FBS	Fetal Bovine Serum
FDA	Food and Drug Administration
FGF-2	Fibroblast Growth Factor-2
FOLFIRI	5-FU, LV and Irinotecan
FOLFOX	5-FU, OX and LV
FTIR	Fourier Transform Infrared
GAE	Gallic Acid Equivalents
GFP	Green Fluorescent Protein

GIT	Gastrointestinal Tract
GML	*Garcinia mangostana* L.
H_3PO_4	Orthophosphoric Acid
HB	Hemoglobin
HCT 116	Human colorectal carcinoma cell line
HEPA filter	High Efficiency Particulate Air filter
HER-2	Human Epidermal growth factor Receptor 2
HIF-1α	Hypoxia Inducible factor -1- alpha
HIFs	Hypoxia Inducible Factors
HIV	Human Immunodeficiency Virus
HPLC	High Performance Liquid Chromatography
HUVECs	Human umbilical vein endothelial cells
I	Intensity
IC50	Inhibition Concentration 50
ICH	International Conference on Harmonisation
IFL	Irinotecan, 5-FU and LV
IFN-α and γ	Interferons alpha and gamma
IGF-I	Insulin-like Growth Factor-1
IgG	Immunoglobulin G
KBr	Potassium Bromide
K-ras/PI3-K	K-ras/Phosphatidylinositol 3-kinases
K-ras/RAF	K-ras/Murine leukemia viral oncogene homolog 1
K-ras/RAL	K-ras/ K-ras related protein

K-ras	Kirsten-rat sarcoma virus
LC-MS	Liquid Chromatography Mass Spectrometry
LD50	Lethal Dose 50
LOD	Limit of Detection
Log D	Partition Coefficient
LOQ	Limit of Quantification
LV	Leucovorin
MAPK/ERK	MAPK-Extra cellular signal regulated enzyme kinase
MAPK/JNK	Mitogen-Activated Protein Kinase/Jun N-terminal Kinase
MAPK	Mitogen-Activated Protein Kinase
MCF-10A	Human epithelial cell from fibrocystic disease
MCF-7	Human hormone sensitive and invasive breast cancer cell line
MCH	Mean Corpuscular Hemoglobin
MCHC	Mean Corpuscular Hemoglobin Concentration
MCV	Mean Corpuscular Volume
MEM	Minimum Essential Medium
Mg	Milligram
MIR	Mid Infrared
MMPs	Matrix Metalloproteinase
mTOR	Mammalian Target of Rapamycin
MTT	Methylthiazolyldiphenyl-tetrazolium bromide
Myc/Max	A signal transduction pathway

N_2	Nitrogen gas
NaCl	Sodium Chloride
NaOH	Sodium hydroxide
NF-KB	Nuclear Factor-Kappa B
NIH	National Institutes of Health
Notch	A signal transduction pathway
Noxa	Damage protein, a pro-apoptotic BH3-containing protein
NPs	Nanoparticles
NSAIDs	Nonsteroidal Antiinflammatory Drugs
OA	Oleanolic acid
OD	Optical Density
OECD	Organization of Economic Cooperation and Development
OX	Oxaliplatin
p53	Tumor suppressor protein 53
PAs	Plasminogen Activators
PBS	Phosphate Buffer Saline
PCS	Photon Correlation Spectroscopy
PCV	Packed Cell Volume
PDGFR	Platelets Derived Growth Factor Receptors
PDI	Polydispersity Index
PE	Plating Efficiency
P-glycoprotein	Permeability glycoprotein

PI3K/AKT/mTOR	Phosphatidylinositol-3-kinase/Protein Kinase B/Mammalian Target of Rapamycin
PIK3CA,	Phosphoinositide-3-kinase, catalytic, alpha polypeptide
PLGA	Poly(lactide-co-glycolide)
PlGF	Placental Growth Factor
PMS	Phenazine Methosulfate
PMs	Physical Mixtures
PNPs	Polymeric Nanoparticles
PPM	Part Per Million
pRB	Retinoblastoma protein
pRb-E2F	A signal transduction pathway
PS	Penicillin/Streptomycin
PUMA	P53 Up-regulated Modulator of Apoptosis
PVP	Polyvinylpyrrolidone
R^2	Regression coefficient
RBCs	Red Blood Cells
RDW	Red Blood cell distribution Width
RLU	Relative Light Units
RSLC	Rapid Separation Liquid Chromatography
R_t	Retention time
S	Slope of the regression equations
SCK	*Syzygium campanulatum* Korth
SD	Standard Deviation

SDH-B, C and D	Succinate Dehydrogenases B, C and D
SDS	Sodium Dodecyl Sulfate
SDs	Solid Dispersions
SEM	Scanning Electron Microscopy
SF	Survival Fraction
SPF	Specific Pathogen Free
SRE	Serum Response Elements
T47D	Human hormone sensitive early stage breast cancer cell line
TCF/LEF1	T-Cell Factor/Lymphoid Enhancing Factor-1
TEM	Transmission Electron Microscopy
T-extract	Toluene extract of *Garcinia mangostana*
TGF-α	Transforming Growth Factor-alpha
TGF-β	Transforming Growth Factor-beta
TGFβR2	Transforming Growth Factor-β II Receptor
TKI	Tyrosine Kinase Inhibitor
TLC	Thin Layer Chromatography
TOF LC-MS	Time of Flight - Liquid Chromatography - Mass Spectrometry
TP53	Tumor protein 53
TRE	Transcription factor Response Elements
TSP-1	Thrombospondin-1
UA	Ursolic Acid

UV-Vis	Ultraviolet-Visible
VEGF	Vascular Endothelial Growth Factor
VEGFR 1 and 2	Vascular Endothelial Growth Factor Receptors 1 and 2
WBCs	White Blood Cells
WHO	World Health Organization
Wnt	Wingless-int, a signal transduction pathway
XRD	X-Ray Diffraction
XTT	3-Bis(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide inner salt

LIST OF UNITS

dL	Deciliter
fL	Fimtoliter
g	Gram
g/dL	Gram/deciliter
h	Hour
Kg	Kilogram
KV	Kilovolt
L	Liter
L/L	Liter/liter
L/min	Liter per minute
m/z	Mass-charge ratio
M	Molar
mA	Milliampere
mg	Milligram
mg/mL	Milligram per Milliliter
min	Minute
mL/min	Milliliter per minute
mL	Milliliter
mm	Millimeter
mM	Millimolar
mV	Millivolt

MΩ	Mega Ohm
ng/mL	nanogram per milliliter
nm	Nanometer
pg	Picogram
RLU	Relative Light Units
rpm	revolution per minute
U/mL	Unit per milliliter
v/v	volume per volume
v/wt	volume per weight
V	Volt
Wt/wt	weight per weight
µg/mL	Microgram/milliliter
µL	Microliter
µm	Micrometer
µM	Micromolar

LIST OF SYMBOLS

α	Alpha
β	Beta
°C	Celsius
γ	Gamma
δ	Standard deviation of the Y intercept of calibration curves
Δψ	Mitochondrial membrane potential
ζ	Zeta potential
λ_{max}	Lambda max
%	Percent
ϴ	Theta

KAJIAN ANTI-KANSER *GARCINIA MANGOSTANA* L. DAN *SYZYGIUM CAMPANULATUM* KORTH TERHADAP KANSER USUS

ABSTRAK

Kajian ini bertujuan untuk menyiasat aktiviti antiangiogenik dan antikanser kolon ekstrak dan isolat-isolat *Syzygium campanulatum* dan *Garcinia mangostana*. Kajian ini juga bertujuan untuk menyediakan ekstrak toluene *G. mangostana.* dalam bentuk nanopartikel dan α-mangostin dalam serakan pepejal untuk meningkatkan kelarutannya dalam akueus. Analisis fitokimia ekstrak *S. campanulatum* secara relatifnya menunjukkan kehadiran asid betulinic dan kandungan polifenol dengan kepekatan yang agak tinggi. Asid betulinik daripada ekstrak *S. campanulatum* tersebut menunjukkan sitotoksik yang poten ke atas sel-sel kanser kolon dengan melalui induksi laluan apoptosis mitokondria. Ekstrak-esktrak *S. campanulatum* tersebut menunjukkan kesan perencatan poten angiogenesis dalam beberapa model *in vitro* dan *in vivo* dengan menghalang ungkapan faktor pertumbuhan endotelial vaskular dan menghalang penghijrahan sel-sel endotelial manusia. Ekstrak metanol menghalang pertumbuhan tumor dalam model xenograft tumor dengan perencatan ketara saluran darah intratumor. α- dan γ-mangostin telah diasingkan daripada kulit buah *G. mangostana*, dan telah dibangunkan untuk menyeragamkan ekstrak-ekstrak *G. mangostana* oleh *UV* spektrofotometri dan kaedah *HPLC*. *Mangostins* terpencil dan ekstrak-ekstrak xanton menunjukkan sitotoksiti poten ke atas sel-sel kanser kolon dengan melalui induksi laluan apoptosis mitokondria, dan juga menunjukkan kesan *in vitro* anti-tumorigenisiti. Sebatian-sebatian itu mendorong aktiviti faktor transkripsi isyarat laluan *p53*, *Myc/Max*

dan *MAPK/ERK* dan menghalang laluan *NF-κB*. Ekstrak toluena *G. mangostana* menunjukkan perencatan pertumbuhan tumor *in vivo* yang ketara dengan permulaan yang lambat. Ekstrak-ekstrak *G. mangostana* dan *S.C.* tidak menunjukkan kesan toksik dan nilai *LD50* dalam tikus *Sprague Dawley* didapati lebih daripada 2000 mg/kg berat badan.

Nanopartikel kationik toluena ekstrak *G. mangostana* telah disediakan melalui kaedah nanopresipitasi dalam polimer *Eudragit RL100* dan *RS100*. Saiz zarah berada di bawah 70 nm dengan kecekapan perangkap tinggi (> 90%). Nanopartikel dikumpulkan dengan kadar hasil >90% oleh pengeringan sejuk beku larutan nanopartikel pukal. Kebolehlarutan ekstrak meningkat secara drastik, dan penghantaran intraselular nanopartikel menyebabkan sitotoksisiti poten kepada sel-sel kanser kolon. Perencatan pertumbuhan tumor *in vivo* telah dicapai pada permulaan awal dalam rawatan nanopartikel berbanding kepada ekstrak yang bukan formulasi. Kepekatan α-mangostin dalam dinding saluran gastrousus haiwan yang dirawat dengan nanopartikel adalah lebih tinggi berbanding dengan yang dirawat dengan ekstrak bukan formulasi. Serakan pepejal α-mangostin dalam *polyvinylpyrrolidone* menghasilkan kebolehlarutan kompaun dengan menggalakkan pemasangan sendiri nanomiseles, pengurangan saiz zarah, dan pembentukan mendakan yang amorfus dan sangat telap. Penghantaran intraselular α-mangostin menyebabkan sitotoksisiti penting kepada sel-sel kanser kolon *in vitro*.

S. campanulatum boleh menyediakan sumber baru asid betulinik dan ekstrak tersebut mungkin mempunyai aplikasi terapeutik untuk pencegahan kemo dan rawatan kanser

kolon. Sistem penyampaian ubat mangostins boleh menjadi aplikasi menarik untuk rawatan kanser kolon.

ANTI-CANCER STUDIES OF *GARCINIA MANGOSTANA* L. AND *SYZYGIUM CAMPANULATUM* KORTH TOWARDS COLORECTAL CARCINOMA

ABSTRACT

This study aims to investigate the anti-angiogenic and anti-colon cancer activity of the extracts and isolates of *Syzygium campanulatum* and *Garcinia mangostana*. And to prepare *G. mangostana* toluene extract in nanoparticles and α-mangostin in solid dispersions in order to improve their aqueous solubility. Phytochemical analysis of *S. campanulatum* extracts reveals relatively high concentration of betulinic acid (BA) and polyphenols. BA fraction from *S. campanulatum* showed potent cytotoxicity on colon cancer cells by inducting the mitochondrial pathway of apoptosis. Extracts also showed potent inhibition of angiogenesis in several *in vitro* and *in vivo* models, by inhibiting the expression of vascular endothelial growth factor and inhibiting migration of human endothelial cells. The methanolic extract also inhibits tumor growth in xenograft tumor model with inhibition of intratumor blood vessels.

α- and γ-mangostin were isolated from *G. mangostana* fruit rinds, and were used to standardize the *G. mangostana* extracts by UV spectrophotometric and HPLC methods. The isolates and the xanthone extracts demonstrated potent cytotoxicity on colon cancer cells by inducing the mitochondrial apoptosis pathway, and also showed effective *in vitro* anti tumorigenicity. The compounds induced the transcription factor activity of p53, Myc/Max and MAPK/ERK signaling pathways and inhibit the NF-κB pathway. The toluene extract of *G. mangostana* showed significant delayed onset inhibition of tumor growth *in vivo*. *S. campanulatum* and *G. mangostana* extracts did not show

severe toxic effects and the LD50 in Sprague Dawley rats was more than 2000 mg/kg body weight. Cationic nanoparticles of *G. mangostana* toluene extract were prepared by the nanoprecipitation method in Eudragit polymers RL100 and RS100. Particle size was below 70 nm with high entrapment efficiency (>90%). Nanoparticles were collected at >90% yield by freeze drying of the bulk nanoparticle solutions. Solubility of extract improved drastically, and the intracellular delivery of nanoparticles caused potent cytotoxicity towards colon cancer cells. Inhibition of tumor growth *in vivo* was achieved at earlier onset in the nanoparticles treatment compared to non-formulated extract. Concentration of α-mangostin in gastrointestinal tract wall was higher in nanoparticles treated animals than in those treated with non-formulated extract. Solid dispersions of α-mangostin in polyvinylpyrrolidone resulted in improved compound's solubility by inducing self assembly of nanomicelles, particle size reduction, and formation of amorphous and highly porous co-precipitates. And intracellular delivery of α-mangostin caused significant cytotoxicity towards colon cancer cells *in vitro*.

S. campanulatum may provide a new source of BA and its extracts may have therapeutic application in colon cancer chemoprevention and treatment. The drug delivery systems of mangostins may find interesting applications in colon cancer treatment.

CHAPTER ONE

LITRATURE REVIEW

1.1 Angiogenesis:

1.1.1 Definition and Role in Physiology and Disease:

Angiogenesis is the formation of new blood vessels from pre-existing vessels. It is a biological process that plays fundamental roles in embryonic development. It also has a critical role in normal physiological situations such as in the female reproductive tract, in the placenta during pregnancy and during wound healing (Auerbach et al., 2003; Folkman, 1995; Vailhe et al., 2001). Angiogenesis is a regulated process while pathological angiogenesis is a deregulated and believed to play a key role in several pathological conditions including proliferative retinopathies, atherosclerosis, rheumatoid arthritis, psoriasis, tumor growth and metastasis (Creamer et al., 2002; Folkman, 1995). Since tumor angiogenesis is essential for growth and metastasis of most solid malignancies, it has became one of the main targets of new cancer therapies (Eatock et al., 2000; Hurwitz et al., 2004; Pfeffer et al., 2003; Scappaticci, 2003). Besides the inhibition of angiogenesis, enhancement of the angiogenesis cascade to stimulate the formation of new microvessels in the myocardium is considered as a target for treatment of ischemic heart diseases (Tabibiazar and Rockson, 2001). Therefore, compounds with either anti-angiogenic or proangiogenic effects could be good therapeutic candidates for the treatment of life threatening diseases such as cancer and ischemic heart diseases.

1.1.2 Angiogenesis - Cascade of Events:

To develop effective angiogenesis modulators, it is crucial to understand the mechanisms underlying the process. Several but tightly regulated steps are involved in the formation of new blood vessels including disintegration of the extracellular matrix

underlying the endothelium, migration, adhesion, and proliferation of endothelial cells, assembly into three-dimensional tubular structure and finally maturation into functional microvessels that support blood flow (Tabibiazar and Rockson, 2001; Papetti and Herman, 2002). Each step can be modulated by several factors and may give the ability to control the process, and hence can be a target for treatment of angiogenesis related diseases.

1.1.3 Regulation of Angiogenesis:

As a tightly regulated process, regulation of angiogenesis depends on the balance between the proangiogenic (stimulators) and the anti-angiogenic (inhibitors) modulators. In their review article, Liekens and his co-workers divided the regulation of angiogenesis into 3 levels; the first is the degradation of the extracellular matrix (ECM), the second is the regulation of angiogenic modulators including the growth factors and the cytokines and enzymes, and the third level is the cell-cell and the cell-matrix interactions (Liekens et al., 2001). The first step in the formation of new vessels is the proteolytic breakdown of the basement membrane underlying endothelial cells in order for them to migrate and invade the stroma of surrounding tissues. This process requires the activity of the plasminogen activators (PAs) and the matrix metalloproteinases MMPs (Mignatti and Rifkin, 1996). The activity of both PAs and MMPs is controlled at their expression level, at the activation level by the proteolytic enzymes, or at the level of their inhibitors such as the tissue inhibitor of metalloproteinase and the inhibitors of plasminogen activators (Liekens et al., 2001).

Subsequent to proteolytic degradation of the ECM and under the influence of a variety of growth factors, the frontline endothelial cells start to migrate through the

degraded matrix and followed by proliferation of their counterparts. Several modulators of angiogenesis including inducers and inhibitors have been described so far including the vascular endothelial growth factor (VEGF), fibroblast growth factor-2 (FGF-2), the angiopoietins 1 and 2 (Ang-1 and 2), angiostatin, endostatin, interferons α and γ (IFN-α and γ) and several other growth factors (Liekens et al., 2001). The regulation of angiogenesis depends on the balance between the stimulators and inhibitors of the process; when the proangiogenic growth factors predominate, then proliferation and migration of endothelial cells is increased and consequently lead to formation of new blood vessels and vice versa.

The cell adhesion molecules, besides the angiogenic enzymes and growth factors, play a critical role in regulation of the angiogenesis cascade of events. Cell adhesion molecules are classified into 4 families including selectins, the immunoglobulin supergene family, cadherins, and the integrins. The integrins for example mediate the interaction of endothelial cells with the ECM during invasion and migration. Also, the cell adhesion molecules are required for cell–cell and cell–ECM interactions which are required for lumen formation and construction of functional capillary loops (Bischoff, 1997).

1.1.4 Tumor Angiogenesis:

Accumulation of somatic mutations causes transformation of normal cells into cancerous cells characterized by uncontrolled proliferation. Consequently, these cells begin to divide rapidly resulting in a microscopically small and localized tumor called *in situ* carcinoma (Folkman, 1995). As the tumor grows, the cells in the tumor center are getting further and further from the main capillary blood supply and thus they suffer the

lack of oxygen and nutrients which can interfere with cell growth leading to cell death. Accordingly, the number of proliferating cells counterbalances the number of dying cells and hence the tumor stops growing and reaches a steady state (Carmeliet et al., 1998). These avascular tumors may remain dormant and undetected for many years with very rare metastasis (Folkman, 1995). After a period of dormancy and as a result of imbalance between the proangiogenic and anti-angiogenic modulators (Giordano and Johnson, 2001; Udagawa et al., 2002), the tumor shifts to the angiogenic phenotype associated with the formation of intratumor capillaries. As a consequence, the tumor grows rapidly and disseminates tumor cells into the blood circulation through the newly formed blood vessels which causes secondary tumor growth in distant locations, i.e. metastasis (Figure 1.1).

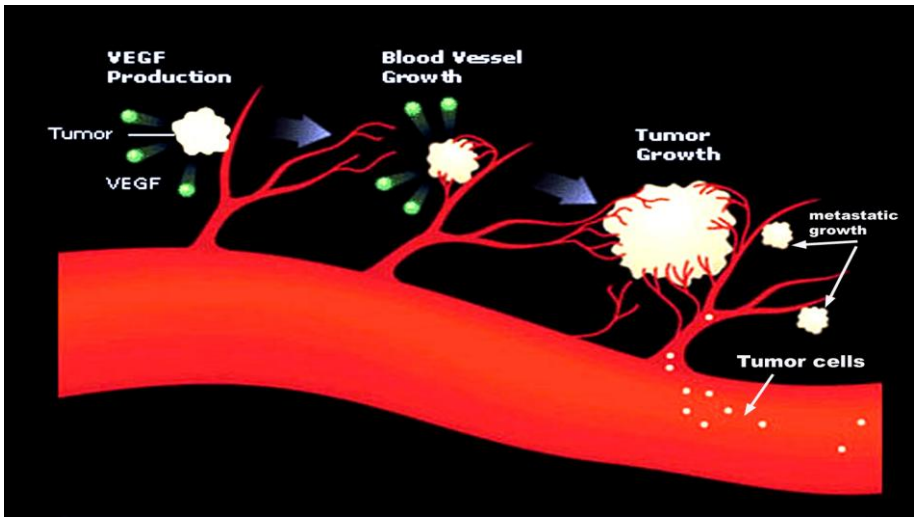

Figure 1.1 Tumor angiogenesis. The figure displays the role of tumor angiogenesis in the growth and metastasis of the primary tumor. Adapted from (Ferrara, 2004).

The angiogenesis phenotype can be triggered by the overproduction of positive regulators of angiogenesis such as VEGF and FGF-2, down regulation of the negative regulators such as the thrombospondin-1 (TSP-1) or by combination of both (Liekens et

al., 2001). Several lines of evidence suggest the central role of hypoxia inducible factors (HIFs) in tumor angiogenesis, metabolism, proliferation, differentiation and metastasis (Rankin and Giaccia, 2008). HIF can directly activate the expression of number of pro-angiogenic factors such as VEGF, VEGF receptors, plasminogen activator inhibitor-1, Ang 1 and 2, platelet-derived growth factor B and MMP-2 and -9 (Hirota and Semenza, 2006). Of those, VEGF-A is particularly the most important due to its potent angiogenic properties, and because it is overexpressed in a large number of human tumors (Dvorak, 2002).

The factors causing upregulation of HIFs were reviewed by Hirota and Semenza (Hirota and Semenza, 2006); of the included factors are hypoxia, and loss of function mutations of succinate dehydrogenases (SDH-B, C and D), mutations in p53 tumor suppressor gene and mutations in the phosphatidylinositol-3-kinase/protein kinase B/mammalian target of rapamycin pathway (PI3K/AKT/mTOR). For example, under hypoxic or even normoxic conditions cancer cells usually obtain their energy from glycolysis rather than oxidative phosphorylation which causes accumulation of lactate and pyruvate. Recent research shows that pyruvate activates endothelial cells and induces angiogenesis by stabilizing HIF-1α (Jung, 2011). The role of the tumor suppressor gene p53 was also addressed which enhances the degradation of HIF-1α and hence the accumulation of somatic mutations in this gene is associated with increased level of HIF-1α and induced tumor angiogenesis (Ravi et al., 2000).

1.1.5 Impact of Tumor Angiogenesis on Tumor Microenvironment:

Newly formed tumor blood vessels often have abnormal structure with markedly increased diameter, convoluted and disorganized architecture. Their walls may have

pores, and are discontinuous or may even lack basement membranes or diminished pericyte coverage making the vessels leaky and often filled with interstitial fluid. And since tumor tissues have abnormal lymph drainage, the interstitial fluid accumulates progressively and consequently the interstitial pressure is increased. As a result, resistance to blood flow is increased that impairs blood supply to the tumor and consequently lead to decreased delivery of nutrients and oxygen and reduced removal of the waste products (Fukumura and Jain, 2007). The lack of oxygen creates hypoxic conditions which in combination with the high rate of anaerobic glycolysis lead to accumulation of acidic byproducts such as lactic acid and increases the acidity of tumor microenvironment (Trédan et al., 2007; Fukumura and Jain, 2007). The reduced intratumor blood flow, reduced supply of nutrients, hypoxia and acidosis are believed to play a critical role in the success or failure of cancer chemotherapy. The reduced intratumor blood blow and the acidic pH hinder the delivery of chemotherapeutics. In addition it causes starvation and diminished oxygen supply that result in reduced proliferation of tumor cells which become quiescent and less responsive to chemotherapy than the rapidly proliferating cells (Trédan et al., 2007). Moreover, the persistent hypoxic conditions creates genomic instability that may cause the selection of more adaptive tumor cells with more aggressive and resistant phenotypes such as those with overexpression of the HIFs, the efflux P-glycoprotein and other survival proteins (Trédan et al., 2007). The acidic environment, besides reducing the delivery of chemotherapies to the growing tumors, inhibits the cellular uptake and hence reduces efficacy of the more alkaline chemotherapeutic drugs such as doxorubicin (Gerweck et al., 2006).

1.1.6 Mechanisms of Anti-angiogenesis Therapy:

Mechanistically, anti-angiogenic agents work by interfering with the key steps of angiogenesis. Regardless of their target molecules, anti-angiogenic therapy such as anti-VEGF may cause vasoconstriction, decreased permeability, and decreased perfusion resulting in decreased delivery of oxygen and nutrients to the tumor (Chi et al., 2009). Theoretically, the constriction of intratumor blood vessels reduces the intratumor blood flow and consequently the delivery of chemotherapeutics is reduced too, which counteracts the reduced supplies of nutrients and oxygen. An alternative hypothesis states that anti-angiogenic therapy may transiently normalize the highly abnormal tumor vasculature and improves the delivery and efficacy of concomitant cytotoxic therapy (Jain, 2005; Ma et al., 2011). According to this hypothesis, anti-angiogenic therapy may work by decreasing the permeability of tumor vasculature and decreasing the accumulation of interstitial fluid which result in a drop of the interstitial pressure and consequently leading to a uniform blood flow and diminished tumor hypoxia (Winkler et al., 2004). When anti-angiogenic therapy is accompanied with cytotoxic chemotherapy, the normalization of tumor vasculature may increase the tumor retention of the cytotoxic agent and hence increasing its efficacy (Ma et al., 2011). The normalization hypothesis is supported by the results of several research groups including (Winkler et al., 2004; Chabot et al., 2011; Hedlund et al., 2009).

1.1.7 Recent Advances in Anti-angiogenesis Therapy:

Since the inhibition of angiogenesis was launched, by Folkman in 1971, as a promising target for the treatment of different tumor types (Folkman, 1971), more than 40 anti-angiogenic drugs have been tested in human cancer patients in clinical trials all over the

world. From functional point of view, these drugs can be classified into 3 groups (Figure 1.2): drugs that inhibit growth of endothelial cells such as endostatin and combretastatin A4, drugs that block angiogenesis signaling such as Avastin® and Interferon-alpha, and drugs that block ECM breakdown such as inhibitors of MMPs. The first class work by inducing apoptosis or inhibiting growth of endothelial cells, the second class inhibits the production of basic fibroblast growth factor (bFGF) and VEGF, whereas the last group works by inhibiting the breakdown of ECM and hence interferes with invasion and migration of endothelial cells. Other new drugs such as the tyrosine kinase inhibitors (Erlotinib, Sorafenib and Sunitinib) block the activity of multiple growth factor receptors such as VEGF and platelets derived growth factor receptors (PDGFR) (Samant and Shevde, 2011).

Seven anti-angiogenic agents have been approved as anti-cancer therapies by the American Food and Drug Administration (FDA). These agents fall in 2 categories including monoclonal antibodies directed against specific proangiogenic growth factors or their receptors, and small molecule tyrosine kinase inhibitors directed against proangiogenic growth factor receptors. Additionally, other molecules with unknown mechanism of action have been also reported such as mTOR inhibitors, proteasome inhibitors and thalidomide (Samant and Shevde, 2011). Another alternative approach in anti-angiogenic therapy is the metronomic chemotherapy i.e. the administration of low doses of conventional chemotherapy at regular intervals. Metronomic chemotherapy schedules work by inhibiting proliferation and survival of tumor endothelial cells, preventing the recruitment of endothelial progenitor cells (EPCs), and have anti-tumor activity (Chi et al., 2009; Samant and Shevde, 2011).

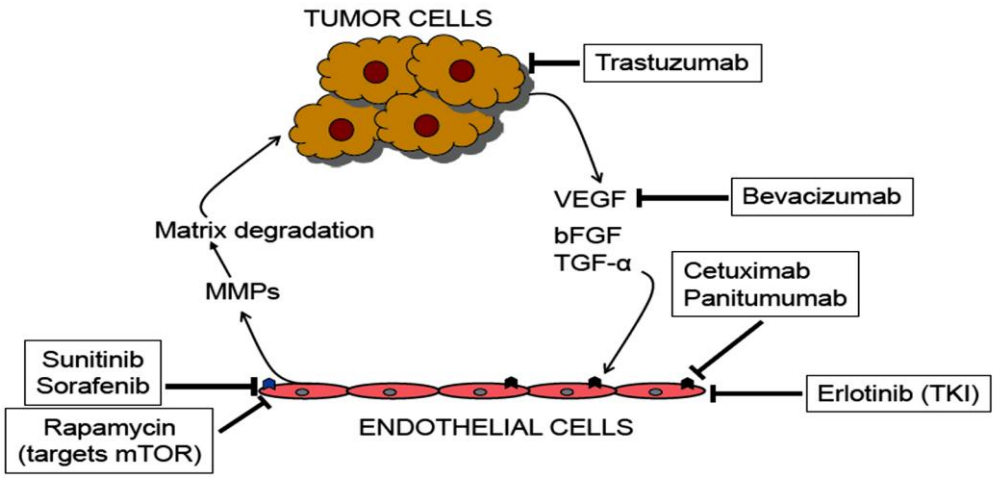

Figure 1.2 Targets of FDA approved angiogenesis inhibitors. Bevacizumab is anti-VEGF antibody that binds to and limits the availability of VEGF. Cetuximab and Panitumumab block the activities of VEGF receptor. Trastuzumab is directed against tumor cells that deprives the effects of HER-2. Erlotinib, Sorafenib and Sunitinib are small molecule tyrosine kinase inhibitors that block the activity of multiple growth factor receptors such as VEGF and platelets derived growth factor receptors (PDGFR). Adapted from (Samant and Shevde, 2011).

Although the monoclonal antibodies have been shown to effectively reduce tumor growth and metastasis, their therapeutic value is still questionable due to the required large doses (20 – 100 mg/kg). In addition, since these recombinant proteins have to be given frequently and on long term basis therefore it seems to be unpractical therapy (Cao, 2004b). Hence, discovery of other alternatives such as small molecules from natural products, for example, seems to be more attractive and have the potential to replace the current anti-angiogenic agents. In this context, polyphenols including catechins, resveratrol, quercetin and many other compounds have been extensively studied and were found to effectively inhibit angiogenesis and tumor growth (Tan et al., 2003; Zhou et al., 2005). Apart having lower side effects, these natural compounds are generally cheaper to produce. With respect to their pharmacokinetics, polyphenols are generally well absorbed after oral ingestion and have relatively long half-life and can be

considered as ideal therapeutics for long-term prevention and treatment of angiogenesis related diseases such as cancer (Cao, 2004a).

1.1.8 Obstacles and Future Perspectives:

Despite the success in some anti-angiogenic therapies, certain issues associated with this type of therapy need to be resolved including efficacy, toxicity, drug resistance and drug delivery to the tumors. Several studies have shown that anti-angiogenic therapy by itself is insufficient to cause shrinkage of pre-established tumors, and recent studies even showed that treatment with anti-angiogenic therapy alone such as Avastin can also enhance the invasiveness of glioblastoma tumor cells (Keunen et al., 2011). Another obstacle that is associated with both chemotherapy and anti-angiogenic therapy of cancer is the abnormal tumor vasculature; solid tumors have impaired blood flow due to the high interstitial pressure which interferes with the delivery of the therapeutic agents to tumor cells. Recently, a study employing animal models showed that anti-angiogenic agents such as Avastin transiently normalize the tumor vasculature and improves the intratumor blood flow and lead to increased retention of the cytotoxic therapeutics (Ma et al., 2011). Therefore, the occurrence of adverse effects when anti-angiogenic therapy is used alone and the improved retention of chemotherapeutics when used in combinations with anti-angiogenic therapy necessitate the combination of the 2 therapeutic regimens in the treatment of cancer.

Previously, it was believed that the clinical use of anti-angiogenic therapy may have fewer side effects than conventional chemotherapy for 2 reasons. First, under normal physiological conditions more than 99% of endothelial cells are quiescent. Second, physiological angiogenesis is distinct from arteriogenesis and

lymphangiogenesis. Therefore, it was proposed that anti-angiogenic therapy may interfere with physiologic angiogenesis associated with wound healing and menstrual cycle and will not cause severe side effects (Wu et al., 2008). However, the results of clinical trials and clinical use of anti-angiogenic therapy in cancer patients revealed the occurrence of severe toxicities including bleeding, delayed wound healing, thrombosis, hypertension, hypothyroidism and fatigue, proteinuria and edema, skin toxicity, leukopenia, lymphopenia, and immunomodulation (Wu et al., 2008). These side effects are caused by the nonselective activity of tyrosine kinase inhibitors and due to inhibition of VEGF pathways that play fundamental roles, either directly or indirectly, in maintaining normal functions of the kidneys, hematopoietic system, skin homeostasis and blood vessels (Wu et al., 2008).

Novel strategies are needed to overcome the toxicities associated with anti-angiogenic therapy. The enhanced permeability and retention effect and the acidic environment, encountered in most solid tumors, can be utilized to achieve selective delivery of cancer therapeutics to tumor cells. The leaky tumor vasculature due to presence of intercellular gaps with 100 – 600 nm (Hashizume et al., 2000), can be employed to passively deliver nanoparticles loaded with the therapeutic agents. Since the enhanced permeability and retention is unique to solid tumors, the nanoparticles delivery systems may be concentrated in the tumor microenvironment where the drug payload will be released. The selectivity of nanoparticles can be improved by active targeting, in which specific ligands to tumor cells such as folic acid can be linked on the surface of nanoparticles which targets them towards tumor cells with overexpression of folic acid receptors. Another targeting approach is by linking the drugs to their carries

via pH sensitive bonds that breakdown in the acidic microenvironment of tumors and therefore drug release will take place only in the acidic microenvironment of solid tumors (Cho et al., 2008). These approaches may help to effectively attack cancer and minimize the side effects due to the nonselective cytotoxicity.

Of the biggest challenges that face anti-angiogenic therapy of cancer is the *de novo* or the acquired drug resistance. Previously it was believed that anti-angiogenic therapy has low likelihood of developing drug resistance since it targets the quiescent and the genetically stable endothelial cells. However, clinical use of anti-angiogenic therapeutics revealed that many tumors initially failed to respond to anti-angiogenic therapy and others develop resistance over time after a period of initial response (Azam et al., 2010).

The mechanisms of resistance are not well understood but basically the presence of a complex network of angiogenesis signaling play fundamental roles in drug resistance. Since most anti-angiogenic therapies are directed towards inhibition of particular targets such as VEGF signaling, the angiogenic cascade can not be totally blocked and hence angiogenic signaling will continue through other alternative signaling pathways (Azam et al., 2010). Several mechanisms of resistance to anti-angiogenic agents have been described including the presence of multiple positive modulators of angiogenesis such as VEGF, placental growth factor (PlGF), upregulation of the hypoxia pathway via stabilization of the HIF-1α, upregulation of proangiogenic stromal cells such as fibroblasts, pericytes and hematopoietic cells that augment tumor vasculature by secreting growth factors such as VEGF, bFGF and MMPs, pericytes regulate proliferation of endothelial cells and hence play a major role in retaining the

vascular integrity after termination of therapy, some anti-angiogenic compounds have hormetic effect i.e. they inhibit tumor growth at high concentration and stimulate tumor growth at lower concentrations, the loss of p53 tumor suppressor gene which plays important role in angiogenesis by modulating the expression of some key players in angiogenesis such as MMPs, overexpression of the membrane P-glycoproteins which work by efflux of the therapeutic agents and hence prevent their accumulation in the cytoplasm of target cells, and finally the tumor endothelial cells are genetically unstable and more resistant than normal endothelial cells (Azam et al., 2010).

Several approaches have been tried to combat the lack of efficacy, toxicity and resistance in anti-angiogenic therapy such as combination of metronomic chemotherapy with anti-angiogenic therapy, the use of liposomes and nanoparticles to achieve selective delivery of the therapeutic agents to tumor cells. Additionally, combination of different anti-angiogenic agents with different mechanisms of action could be also tried, however it should be applied in the form of active drug delivery in order to diminish the nonspecific effects and hence to reduce the side effects.

1.2 Colorectal Carcinoma:

Colon cancer is a multi-step disease that occurs as a consequence of a series of pathologic changes that transform normal epithelial cells of the colon into invasive carcinoma (Figure 1.3). Several studies showed that this multi-step process is accompanied by specific mutations including the adenomatous polypsis coli (APC) gene, cyclooxygenase-2 (COX-2), (Kirsten-rat sarcoma virus) K-ras mutations, loss of the 18q21 gene, microsatellites instability and mutations in transforming growth factor-

β II receptor (TGFβR2), and stabilization and translocation of β-catenin (Gustin and Brenner, 2002; Kobayashi et al., 2000; Kanwar et al., 2010).

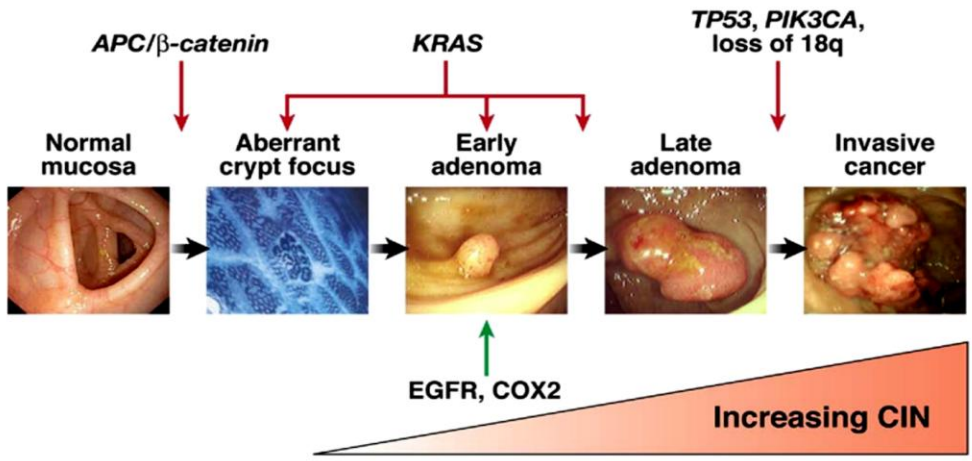

Figure 1.3 Stages of colon carcinogenesis. The figure shows the stages of colon cancer and the molecular changes that accompanied each stage, these changes can be considered as targets to prevent colon carcinogenesis. Adapted from (Pino and Chung, 2010).

The role of the APC suppressor gene in the early stages of colorectal carcinoma was extensively studied. Loss of function mutations of the APC gene is associated with accumulation of intracellular β-catenin which plays a role in cell adhesion and acts as a transcription factor of the Wnt signaling pathway (Henderson, 2000). Activation of Wnt/β-catenin signaling pathway leads to activation of T-cell factor/lymphoid enhancing factor-1 (TCF/LEF1) transcription factors and subsequently to expression of several target genes including c-Myc, cyclin D1 and COX-2 that have been implicated in tumorigenesis of colorectal carcinoma and several other cancers (Gehrke et al., 2009).

Cyclooxygenases (COXs) exist in 2 isoforms COX-1 and COX-2 and several studies have shown that COX-2 is overexpressed in human colon cancer and adenomas.

COX-2 converts arachidonic acid to prostaglandins which may stimulate proliferation of cancer cells, inhibit apoptosis and/or stimulate angiogenesis. Also the prostaglandins were found to work by inducing the production of some growth factors such as VEGF and hepatocyte growth factor that induce tumor angiogenesis and proliferation of the tumor cells (Ota et al., 2002).

The role K-ras proto-oncogene in tumorigenesis of colon cancer has been extensively studied. K-ras proteins are located on the inner layer of plasma membrane and can be stimulated by extracellular signals such as growth factors and cytokines. In normal physiological conditions the activated K-ras proteins are inactivated after short period while their mutant counterparts are constitutively activated and continue to activate their downstream effector proteins. Activity of K-ras is mediated via different signaling pathways including K-ras/RAF, K-ras/PI3-K and K-ras/RAL that are involved in regulation of proliferation, survival, invasion and metastasis, and angiogenesis (Castagnola and Giaretti, 2005). Oncogenic mutations in K-ras were found to play central roles in tumorigenesis of colorectal carcinoma and their presence indicates poor prognosis (Conlin et al., 2005).

Other types of mutations are also involved in colon carcinogenesis including the chromosomal and microsatellites instability. The chromosomal instability results from a series of genetic alterations that involves activation of oncogenes such as K-ras and inactivation of tumor-suppressor genes, such as TP53, APC and deleted in colorectal carcinoma gene (DCC) located on chromosome 18q21, these mutations are associated with increased risk of relapse and death among colon cancer patients (Pino and Chung, 2010). Whereas the microsatellites instabilities are caused by deficient DNA mismatch

repair and consequently lead to increased mutation frequency particularly in the repetitive microsatellite sequences (Gervaz et al., 2002; Castagnola and Giaretti, 2005). Frameshift mutations of TGFβR2 are encountered in more than 80% of colon cancer patients with microsatellite instability which make tumor cells more resistant to anti-tumorigenic effects of TGF-β, thus inhibition of the TGF-β signaling pathway may contribute to the formation of primary colon tumors. *In vitro* experiments showed that inactivation of TGFβR2 in HCT 116 cells is associated with increased proliferation rate, and *in vivo* work indicated that mutant TGFβR2 may contribute to transformation of colorectal carcinoma (Grady et al., 2006).

1.3 Major Signaling Pathways in Colon Carcinogenesis:

1.3.1 Wnt/β-catenin Signaling Pathway:

The Wingless-Int. (Wnt) signaling pathway plays a fundamental role in embryonic development, in regeneration of tissues and has a key role in cell-cell signaling (Logan and Nusse, 2004). Mutations in the Wnt pathway play a crucial role in cancer development and particularly in colon cancer. Almost 90% of all colon cancer cases are associated with mutations of APC tumor suppressor gene resulting in accumulation of Wnt/β-catenin (Giles et al., 2003). β-catenin complexes with TCF/LEF transcription factors and subsequently enhances the expression of downstream effector genes such as c-Myc, cyclin D1 and MMP genes which are involved in carcinogenesis and tumor angiogenesis (Gehrke et al., 2009). Therefore, downregulation of the Wnt pathway could be potential target in the treatment of various types of cancer including colon cancer.

1.3.2 Notch Signaling Pathway:

Notch signaling pathway regulates the development of the central nervous system, the cardiovascular system, the endocrine system, bone development and tissue renewal. Notch signaling controls a range of cellular functions in normal physiological and pathological conditions including cell fate specification, differentiation, proliferation, apoptosis, adhesion, migration and angiogenesis (Bolos et al., 2007). Depending on signal strength, timing, cell type and the normal function of a given tissues, Notch can act as an oncogene or a tumor suppressor gene (Maillard and Pear, 2003). Recent studies in mutant APC mice showed that both of Wnt and Notch signaling pathways are activated which indicate that Notch may function downstream of the Wnt pathway in the intestine (Radtke and Clevers, 2005). Therefore, Notch signaling may provide an alternative therapeutic target and could be used in combination with Wnt inhibitors for treatment of colorectal carcinoma.

1.3.3 P53 Signaling Pathway:

The p53 tumor suppressor gene plays a fundamental role in cell cycle regulation and apoptosis. The activation of this pathway is associated with either cell cycle arrest or induction of apoptosis which depends on the strength and frequency of the signal (Haupt et al., 2003). Mutations in p53 suppressor gene are common in all cancers and loss of function mutations are encountered in more than 60% of cancer patients and associated with decreased sensitivity to chemotherapeutics (Machado-Silva et al., 2010) . p53 controls cell death by regulation of genes involved in both the extrinsic and intrinsic pathways of apoptosis either via transcriptional dependent or transcriptional-independent mechanisms (Yu and Zhang, 2005). Therapeutic manipulation of p53

pathway is a promising strategy in cancer treatment and is expected to target a broad range of cancers with more selectivity and lower side effects. Several approaches including the use of small peptides or small molecules have been tried in order to reactivate the suppressed wild-type p53 or reverse the mutant into a wild-type p53(Machado-Silva et al., 2010).

1.3.4 TGF-β Signaling Pathway:

TGF-β signaling pathway has dual effects; tumor suppressor effects in the early stages of tumor development where it inhibits tumor progression by inducing apoptosis of tumor cells, and oncogenic effects in the late stages of tumorigenesis where it inhibits apoptosis, enhances metastasis and invasion of tumor cells, and provokes tumor angiogenesis (Sánchez-Capelo, 2005). In colorectal carcinoma, frameshift mutations in TGFβR2 are frequently encountered in more than 80% of cases with microsatellite instability. Inhibition of the TGF-β signaling pathway make tumor cells more resistant to anti-tumorigenic effects of TGF-β and may contribute to the formation of primary colon tumors (Grady et al., 2006). However, the opposing effects of TGF-β signaling pathway hold back the interest in this pathway as a therapeutic target of cancer.

1.3.5 Cell Cycle (pRB/E2F) Signaling Pathway:

Mutations that affect the retinoblastoma cell cycle signaling have been documented in nearly every type of adult cancer (Sellers and Kaelin, 1997). The retinoblastoma tumor suppressor gene encodes a protein called pRB which binds with a series of transcription factors termed E2F and forming dimmers that regulate the expression of several downstream effector genes involved in cell cycle control, DNA licensing and synthesis, mitosis, DNA repair and apoptosis (Stevaux and Dyson, 2002). The E2F transcription

factors were found to have both tumor suppressor and oncogenic effects which can be determined by the presence of active pRB suppressor gene. The concentration of E2F is increased in the presence of aberrant pRB and consequently increases cell proliferation and inhibits apoptosis via downregulation of p53. On the contrary, DNA damage which in the presence of wild-type gene can either induce cell cycle arrest or apoptosis, depending on the extent of DNA damage; pRB binds to the repressor E2Fs and induces G1 arrest and consequently DNA repair, or it binds to activator E2Fs where it induces apoptosis via activation of caspases 3 and 7, p53 and Apaf-1 and downregulation of the antiapoptotic proteins such as Bcl2 (Tsantoulis and Gorgoulis, 2005).

1.3.6 NF-κB Signaling Pathway:

The nuclear factor κB (NF-κB) represents a family of transcription factors that play a critical role in regulation of various biological processes such as immune and inflammatory responses, cell growth, migration, adhesion and apoptosis (Sun and Xiao, 2003). NF-κB can be activated by several stimuli such as inflammatory cytokines, growth factors, DNA damaging agents, bacterial components and viral proteins (Pahl, 1999). In normal physiological conditions NF-κB is only transiently activated and its deregulated activation has been encountered in a large variety of human malignancies such as leukemia, breast cancer, colon cancer, ovarian cancer, prostate cancer, liver cancer and melanoma (Sun and Xiao, 2003). Besides its role in oncogenesis, the NF-κB also plays crucial role in resistance of tumor cells to anti-cancer therapies (Scartozzi et al., 2007). The constitutive activity of NF-κB can be caused by genetic alterations in genes encoding NF-κB itself, or due to constitutive activation of the NF-κB-activating kinase. Increasing numbers of studies showed that inhibition of NF-κB can induce

apoptosis of tumor cells and hence NF-κB inhibitors are expected to enhance the anti-tumor efficacy of chemotherapeutics (Sun and Xiao, 2003).

1.3.7 Myc/Max Signaling Pathway:

Myc is a transcription factor with dual effects; from one side it is required for the progression of cell cycle in normal cells and its overexpression in cancer cells acts as angiogenic switch. On the other hand, Myc/Max heterodimers induce intracellular transduction pathways required for induction of apoptosis (Nilsson and Cleveland, 2003). Myc dimerizes with its partner protein Max and consequently binds to DNA where the complex modulates expression of the target genes including p53 and the mitochondrial proapoptotic proteins that work by enhancing release of cytochrome c and induction of apoptosis (Yang et al., 2009)

1.3.8 Hypoxia Signaling Pathway:

Hypoxia inducible factor is a master transcription factor that controls nutritional stress, angiogenesis, tumor metabolism, invasion, autophagy and cell death (Pouysségur et al., 2006). Several studies showed that HIF-1α and HIF-2α are overexpressed in primary and metastatic human cancers and are associated with tumor angiogenesis and poor prognosis (Semenza, 2003). As solid tumors grow, the tumor mass in the center becomes far from oxygen and nutrients supply. Low concentration of oxygen activates HIF indirectly via specific cellular enzymes that sense the variations in oxygen tension (Po_2) (Berra et al., 2006). Activation of HIF is a multi-step process that involves stabilization, nuclear translocation, heterodimerization, transcriptional activation and interaction with other proteins (Brahimi-Horn et al., 2005). In the nucleus, HIF binds to hypoxia-response elements where it regulates the expression of about 100 genes

including activation of genes involved in angiogenesis such as VEGF-A and Ang-2, activation of genes involved in cell invasion, migration and metastasis such as MMP-2, urokinase plasminogen activator receptor and E-cadherin, and inhibition of the m-TOR pathway (Pouysségur et al., 2006). These genes are associated with excessive tumor angiogenesis, metastasis and induction of autophagy and hence the tumors become more adaptive with nutrient and oxygen deprivation and more resistant to chemotherapy (Melillo, 2007; Semenza, 2003). The critical role of HIF in development of primary and metastatic tumor growth makes it as a good target in cancer therapy. However, its fundamental role in inflammation may hamper this strategy as the prolonged use of HIF-1 inhibitors is associated with severe immunodeficiency. Thus, it seems more attractive to target the HIF gene products rather than targeting HIF itself (Pouysségur et al., 2006).

1.3.9 MAPK Signaling Pathways:

The mitogen-activated protein kinases (MAPKs) control many and fundamental physiological functions. MAPKs are sub-divided into 4 sub-groups including the extracellular regulated kinase 1 and 2 (ERK 1 and 2), ERK5, p38 MAPKs and the c-Jun amino terminal kinase (MAPK/JNK) (Shen et al., 2001; Johnson and Lapadat, 2002). MAPKs signaling play fundamental roles in cell proliferation, differentiation, survival and cell death (Chang and Karin, 2001; Qiao et al., 2001). The role of MARK/ERK pathway has been extensively studied and devastating lines of evidence indicate an oncogenic, mitogenic, and prosurvival roles and hence it is believed that MAPKs have a central role in development of some types of cancer such as colon and malignant melanoma (Davies et al., 2002). So far inhibitors of MAPK/ERK pathway are

considered as potential therapeutics of several types of cancer. On the other hand, some evidence suggests that the activation of MAPK/ERK pathway rather than its inhibition induced cell cycle arrest and/or apoptosis and therefore may provide a therapeutic target of different types of cancer such as small cell lung carcinoma (Ravi et al., 1998), osteosarcoma (Yang et al., 2008) and pancreatic cancer (Sahu et al., 2009). Furthermore, activation of the MAPK/ERK pathway is implicated in inducing apoptotic effects as a consequence of DNA damage caused by cisplatin (Wang et al., 2000), etoposide (Stefanelli et al., 2002), doxorubicin, and ionizing and ultraviolet irradiation (Tang et al., 2002).

The mechanism of the MAPK/ERK mediated apoptosis was recently reviewed by Cagnol and Chambard (Cagnol and Chambard, 2010); both the intrinsic and extrinsic pathways of apoptosis can be induced by the activated ERK that depends on the nature of the treatment and cell type. Moreover, ERK pathway was found to induce cytochrome c release by modulation of the Bcl2 protein family and more specifically by downregulation of the antiapoptotic proteins and upregulation of the proapoptotic proteins. In addition to the direct effect on the apoptotic mediators, activation of ERK pathway is associated with increased stability and activity of p53, and increased stability of c-Myc which in turn increases the proapoptotic effects of p53.

1.4 Colon Cancer Chemotherapeutics Strategies:

To date, no curative therapy is available for colon cancer and most types of cancer as well and the available treatments are meant to prolong the disease-free interval. Treatment of colorectal carcinoma is based on the use of cytotoxic agents including 5-fluorouracil (5-FU), oxaliplatin (OX), irinotecan, leucovorin (LV) and capecitabine

(Cap). Different combinations of these agents were extensively studied in phase II and phase III clinical trials such as IFL (irinotecan, 5-FU and LV), FOLFOX (5-FU, OX and LV), FOLFIRI (5-FU, LV and irinotecan) and CapOx (capecitabine/oxaliplatin). All of these combinations showed better therapeutic outcome than the monotherapy (Cercek and Saltz, 2008). After the development of the monoclonal antibodies bevacizumab (anti-VEGF), panitumumab (human anti-EGFR) and Cetuximab (chimeric human-mouse anti-EGFR), several combinations of these agents with the cytotoxic drugs have been studied in phase II and phase III clinical trials. In general, the results showed that combination of either anti-VEGF or anti-EGFR antibodies with the cytotoxic agents resulted in better therapeutic outcome than each individual therapy. However, the combination of anti-VEGF and anti-EGFR with irinotecan was found to have a negative impact on the therapeutic outcome which may depend on the molecular status of the tumor such as the presence of wild-type or mutant K-ras (Cercek and Saltz, 2008).

1.5 Polymeric Nanoparticles as Drug Delivery Systems:

Polymeric nanoparticles (PNPs) represent one of the promising controlled-release drug delivery systems that can be utilized in the treatment of different human diseases. The controlled-release drug delivery systems are aimed to enhance bioavailability, improve efficacy, reduce toxicity, and improve patient compliance and convenience, and hence are more advantageous than the conventional dosage forms.

1.5.1 Definition of Nanoparticles:

NPs including the nanospheres and nanocapsules are often defined as solid colloidal particles in the range of 10 – 1000 nm. Where the nanospheres are solid matrices whose