Novel Molecular Oncotargets and Nano-Oncotherapeutics

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Edited by

Biswajit Mukherjee

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I dedicate this book to my beloved parents, whose love, affection, support, and blessings have always instilled in me to be blessed with perseverance, dedication, devotion, confidence, and sincerity.

To my beloved wife, and lovely son and wonderful daughter for their continuous love, endless support, and constant encouragement, without which it would be difficult for me to complete the book

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PREFACE

The malignant variety of cancer is the uncontrolled cellular proliferation that produces abnormal cells with many genetic alterations, spreads to other tissues and organs (metastasis), and destroys normal cells. Such proliferation with the typical cellular structure limiting in a particular area with slow growth and generally harmless characteristics forms benign tumor. However, the deadly malignant form of cancer is the second prime cause of death globally and is a significant concern.

The word 'cancer' was coined in 1600 BC. Since then, cancer patients/ cases of cancer have been ever-increasing. New terms such as oncology, metastases, and knowledge related to the causes, such as genetic mutation, have come into being. Despite so many efforts of different treatment modalities, no satisfactory answer for a cancer cure has been achieved. The emergence of nanotechnology and the products obtained from it have established their capability and reckon lots of hope and expectations in various fields, including medicine. Using it in drug delivery can bring a new therapeutic avenue for managing several diseases, including cancer. Further research on new drug targets in cancer should be explored more extensively, and nanotherapeutics should be utilized to achieve more satisfactory therapeutic approaches to cancer control.

Cancer kills globally nearly 10 million people every year. Surgery, radiotherapy and chemotherapy cannot yet provide any curative solution of cancer. Further, metastasis complicates cancer by spreading it at different tissues. However, researchers have explored many new molecular mechanisms of cancer progression. Simultaneously, new therapeutic strategies have been investigated. Nanotherapeutics and nanotheranostics have emerged as a powerful therapeutic tool to develop highly efficacious precision medicines to localize the drug at the target site. Such target–specific drug delivery systems are needed because they can decrease dose and frequency of administration of drugs and thereby, reduce drug-related toxicity. The integrated diagnostic and therapeutic function together in nanotheranostics makes it incredibly beneficial and extremely attractive for cancer therapy. It could be an ideal approach of cancer therapy, that is, “search, spot and kill” the cancer cells. This book will cover the advancements of molecular mechanism in oncology to identify new

therapeutic, prognostic and diagnostic targets in the molecular events of neaplasia and their modulation through novel nanotherapeutics and nanotheranostics toward cure or further delay of the various cancerous processes.

The book has described two aspects that explore molecular therapeutic targets of cancer and cancer control by nanotherapeutics. The first six chapters have highlighted cancer cell proliferation and differentiation, innate immunity, the cancer microenvironment, and the potential therapeutic targets in significant cell signaling pathways, autophagy-associated molecular therapeutic targets, and apoptosis-associated potential therapeutic targets to develop cancer therapy. The following chapters have dealt with cancer prognosis, diagnosis, and therapy, by applying nanotechnology in cancer prevention and therapeutics. Novel strategies for targeted nanotherapeutics for cancer control, chemical stimuli responsive nanotherapeutics for cancer management, nanocarriers for delivery of cancer therapeutic agents, and polymersomes for delivery of nanotherapeutics for cancer control are some of the areas described vividly in this book. The chapters focused on the ethics and nanotechnology in therapeutics, recent patents and patent applications on cancer nanotherapeutics and nanotheranostics, and future perspectives of nanotherapeutics in cancer treatment have enriched the book, too.

The book content is a timely topic to explore the best option for the therapeutic management of cancer that may show a steady reduction of cancer cases worldwide. This book describes new and vital therapeutic approaches to cancer therapy for effective cancer control in patients. This book is helpful for graduate and postgraduate students, researchers, and teaching faculties studying and working in this area.

I express my thanks and gratitude to all the contributors who devoted their time and sincere efforts to write the chapters for the book.

Professor Biswajit Mukherjee
Department of Pharmaceutical Technology
Jadavpur University
Kolkata 700032, India

CHAPTER ONE

CANCER AT A GLANCE, CELLULAR PROLIFERATION AND DIFFERENTIATION

DEBANJALI DASGUPTA[1]
AND SOMNATH DATTA[2]

Abstract

Cancer is the second most important cause of death worldwide. Recently, with changes in regular habits and lifestyles, cancer occurrence is increasing unlimitedly and requires substantial attention. The risk factors or carcinogens behind cancer are categorized into physical, chemical, and biological, based on their origin. Cancer development occurs via several steps. First, some cells lose growth control due to gain and loss of functional mutations of proliferation-related genes or tumor suppressor genes, followed by the clonal selection of those mutant cells with higher proliferation and survival capability. Increased cell division results in the formation of cell mass termed a tumor. Tumors are of two types based on their properties: benign (non-cancerous) tumors and malignant (cancerous) tumors. Benign tumors are composed of abnormally divided cells that do not have the property of invading other unrelated neighboring tissues or organs of the body. Malignant tumors can metastasize to distant organs of the body, invades the tissue, and build a secondary tumor at the new site. Loss of cell-to-cell-junction enhances angiogenesis, and lymphangiogenesis promotes cancer development, supplying adequate oxygen and nutrients to the cell mass. Cancer cells lose their differentiation property and behave like undifferentiated primitive ones. Loss of differentiation marks cancer to be more aggressive than a well-

[1] *Physiology and Biomedical Engineering, Mayo Clinic, Rochester, MN, USA*
[2] *Duke Molecular Physiology Institute (DMPI), Duke University, School of Medicine, Duke University, North Carolina, USA.*

differentiated one. The recently developed therapeutic approaches mainly target cell differentiation-related molecules to aggravate their function, promote cellular differentiation, and terminate malignancy.

Keywords: Cancer, Mutation, Tumor suppressor, Metastasis, Angiogenesis, Anaplasia.

1. Introduction

In multicellular organisms, including humans, cellular proliferation is indispensable to maintain fundamental physiological processes, including embryonic development, organ, and tissue growth, wound healing, and regular biological activities. In biology, cellular proliferation indicates 1) Cell division: The division of a parent cell into daughter cells, thereby increasing cell numbers, and 2) Cell growth: An increase in the size of the cell by the accumulation of cell mass. Continuous quality control removes damaged or dead cells from the physiological system. Overall, cellular proliferation is tightly orchestrated by intracellular gene regulatory networks and extracellular growth factors to maintain a homeostatic balance between continuous cell division and cellular loss due to death. However, once that genetic network disrupts, the entire orchestra crashes, causing uncontrolled, abnormal cell division, often forming a cell lump in solid tissues, such as neoplasm or tumor. Based on their characteristics and ability to spread, neoplasm or tumors are classified into two distinct classes: A) Benign tumors: They have the following characteristics. Mass of abnormal cells grow faster than their neighbor cells and do not die when they should. The growth rate is slower than other tumors and maintained within certain limits; the basal membrane remains intact at a cellular level. The cells lack invading property as they stay at their primary location and cannot spread to nearby tissue or a distant body part (Fig. 1-1A). Examples of benign tumors include benign bone tumors, gland tumors, adenomas, meningiomas, fibroids, lipomas, etc. B) Malignant or cancerous tumors: Cancerous tumors are masses of genetically mutated cells that multiply and form a population of cells with a similar genetic mutation(s). In the cancer population, the basal membrane is fragmented, disrupted, and sometimes disappears due to changes in its structure and various components. Sometimes, secretion of collagenase type IV by malignant cells destroys type IV collagen of neighbor cells resulting in the lysis of basal membranes of those neighbor cells. It helps in the metastasis of the cancer cells by invading and colonizing the territory of neighboring cells

or tissue. Malignant cells also lose their differentiation property (Fig. 1-1B).

Examples of these malignant cell types include all sarcomas, carcinomas, lymphomas, etc. Another tiny group of precancerous or premalignant tumors is defined as the mass of cells that can become malignant if left untreated. Examples: Keratosis, dysplasia, colon polyps.

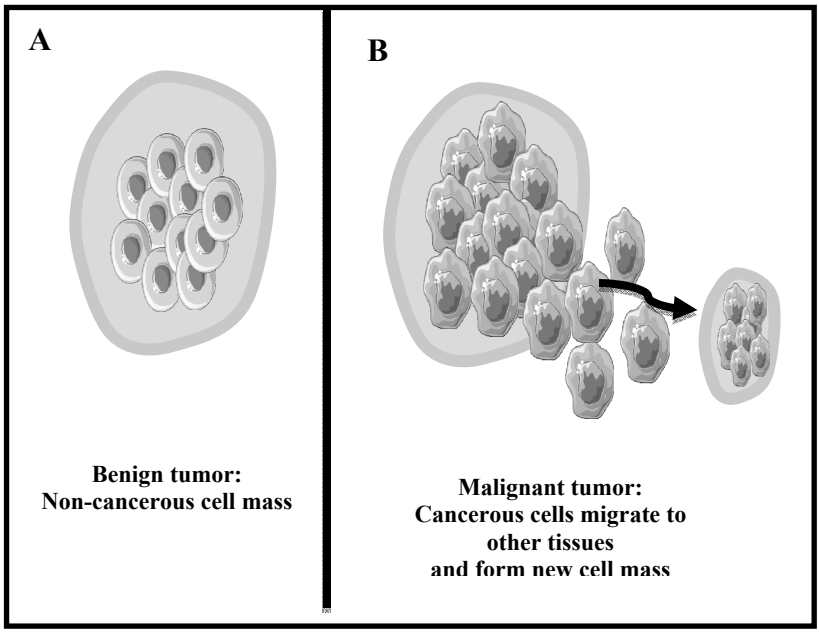

Fig. 1-1 (A, B). Benign tumor vs. malignant tumor

This chapter discusses the epidemiology of cancer, different carcinogens and their role in cancer; cell proliferation in cancer and different factors responsible for cell proliferation; metastasis, invasion, and angiogenesis in cancer; and cell differentiation in cancer.

2. Epidemiology of cancer - at a glance

Cancer, one of the major causes of worldwide mortality, is reported to be responsible for the death of about 10 million people in 2020 (Wild, Weiderpass, and Stewart, World Cancer Report 2020, 16). According to

the National Cancer Institute (NCI), the death rate of cancer is 158.3 per 100,000 people per year (2013–2017 death report by NCI). Cancer-related mortality exhibits more sex bias toward males than females (189.5 per 100,000 male subjects vs. 135.7 per 100,000 female subjects). Cancer is the first or second most common cause of mortality in most countries before age 70 (Wild, Weiderpass, and Stewart, World Cancer Report 2020, 16). In 2020, due to the pandemic outbreak of coronavirus, reduced access to hospitals and clinics adversely affected the diagnosis and treatment of cancer. Ultimately, there was an apparent reduction in the incidence rate due to the delay or absence of a diagnosis, followed by a surge in disease advancements and enhanced mortality (Englum, Prasad, Lake, Mayorga-Carlin, *et al.* 2022, 1048-1052).

In recent years, the rate of cancer occurrence has been increasing rapidly, mainly due to changes in lifestyle and other associated habits. As per the statistical cancer survey by NCI (2017), 39.5% of the human population is expected to be diagnosed with cancer during their lifetime (based on 2015–2017 data). Interestingly, in the United States, about 16.9 million people were estimated as cancer survivors as of January 2019, which is expected to increase to about 22.2 million by 2030.

The causes or risk factors for cancers can be categorized as mentioned below:

a) **Physical carcinogens** are different types of radiation, including ultraviolet radiation that comes from sunlight, industrial radioactive materials, and ionizing radiation from X-rays (Irigaray and Belpomme 2010, 135-148).
b) **Chemical carcinogens** include asbestos, nickel, cadmium, vinyl chloride, alcohol, aflatoxin, a well-known food contaminant, arsenic (a drinking water contaminant), benzidine, benzene, tobacco smoke, and several types of air pollutants.
c) **Biological carcinogens** include viral, bacterial, or parasitic infections that can lead to cancer development. In 2018, biological carcinogen infections contributed to approximately 13% of globally diagnosed cancers (Helicobacter pylori, human papillomavirus (HPV), hepatitis B virus or HBV, hepatitis C virus or HCV, and Epstein-Barr virus) (Irigaray and Belpomme 2010, 135-140). Besides their (Biological carcinogens) direct effect on cellular damage, these biological carcinogens enhance the risk of other cancer developments, i.e., infection with HBV and HCV increases

the risk for liver cancer (Ringelhan, McKeating, and Protzer 2017, 1-3). HIV infection increases the risk of cervical cancer development six-fold (Stelzle, Tanaka, Lee, Khalil, *et al.* 2021, e161-e163).

According to a recent report published by WHO in 2020, breast cancer serves as the most common one with the new cases (2.26 million cases); followed by lung cancer (2.21 million cases); colon and rectal cancer (1.93 million cases); prostate cancer (1.41 million cases); skin cancer (non-melanoma) (1.20 million cases) and stomach (1.09 million cases) for that year. The same report suggested that lung cancer caused most deaths (1.80 million deaths), followed by colon and rectal cancer (916 000 deaths); liver cancer or hepatocellular carcinoma (830 000 deaths); stomach cancer (769 000 deaths); and breast cancer (685 000 deaths) for the year of 2018.

3. Normal cell to cancer cell: overview

Normal cells are the fundamental units of a living body. These cells proliferate or divide to produce new daughter cells, grow, and differentiate between developing proper phenotypical and functional characteristics, getting old, and dying when the death signal comes, termed programmed cell death. The loss of this orchestration leads to the normal cells becoming cancerous. The signal comes from the DNA of the cells first. Mutation in the genes that code for the cell cycle regulatory proteins leads to the uncontrolled proliferation of the cells and evades the regular cell death phase. The uncontrolled division of the cells leads to cell mass or tumor formation. Cancerous cells, unlike normal cells, start invading nearby tissues, spread to distant areas of the body, and form a secondary tumor. It is associated with the formation of new blood vessels at the tumor site to provide oxygen and nutrients to the mass of cells. Another hallmark of a cancer cell is the loss of its differentiation property. More aggressive cancers are characterized by less differentiated cancer cells that are phenotypically and functionally different from normal cells. The stages of cancer development are described in the following paragraphs.

4. Sustained cell proliferation in cancer

Sustained cell proliferation is a significant feature in the development and progression of cancer. The first and foremost step of cancer development is the uncontrolled and continuous random proliferation of cancer cells. Normal cells control their proliferation rate by responding correctly to the

signal for proliferation and maintaining their normal phenotype and function, whereas cancer cells grow and divide in an uncontrolled way as the cells genetically lose growth control and start behaving differently than their neighboring non-cancerous cells. Cancer cells possess a unique characteristic that allows them to survive beyond their average life expectancy and undergo uncontrolled proliferation (Feitelson, Arzumanyan, Kulathinal, Blain, *et al.* 2015, S25-S30). Carcinogenesis, or oncogenesis, or cancer development and progression, is a multi-step process. The evolution of cancer is characterized by the clonal expansion of single cells followed by clonal selection. At the very beginning, cells start accumulating genetic mutations of the growth control and proliferation-related genes, followed by the selection of the cells with gradually increasing capacity for proliferation and survival (Cooper 2000, 571-581). Different stages of abnormal cell proliferation in cancer are described below.

A. Genetic mutation and cancer

Somatic mutation in the DNA serves as the primary precursor for cancer development (Brücher and Jamall 2016, 1663-1665). With the recent developments in whole genome sequencing analysis (GWAS), the genome sequence of distinct types of cancer has become feasible. All available findings support the involvement of the convenient central dogma. Cancer genome mutations can be classified into several types according to their consequences and contributions to cancer development. Mutations in the genes that control the cellular proliferation, growth, differentiation, and survival of cancer cells and directly affect oncogenesis are collectively known as driver mutations (Brown, Li, Goncearenco, and Panchenko 2019, 1-5; Pon and Marra 2015, 25-30; Bozic, Antal, Ohtsuki, Carter, *et al.* 2010, 18545-18550). These mutations are selected evolutionary for clonal expansion in the tumor microenvironment as it confers the growth advantages found in cancer (Greaves and Maley 2012, 306-309). After the acquisition of driver mutation by cancer cells, several random mutations accumulate inside the cancer cell genome without having any structural or functional contribution to the cancer development and progression, which are named passenger mutation (Stratton, Campbell and Futreal 2009, 719-721; Pon and Marra 2015, 25-30). Another kind of mutation(s) impairs cell survival and promotes cell death. These mutations undergo negative selection and are not incorporated into the cancer genome (Stratton, Campbell, and Futreal 2009, 721).

B. Cancer cells escape the controlled cell cycle phases

Somatic cells in the body undergo a well-regulated cell cycle and pass the genetic material to the daughter cells. However, interphase and mitosis, two main cell cycle phases, are accompanied by several checkpoints (Barnum and O'Connell 2014, 29-30). In brief, after a complete cell division, the new two cells enter the resting phase known as the G1 phase, where cells acquire all the necessary mediators for DNA replication and proceed to the next S phase. The DNA gets replicated in the S phase to make sister chromatids. The cell then proceeds into the second resting state named G2 to prepare further for cell division and recheck the duplicate chromosomes for errors. Errors are also repaired before the subsequent mitosis (M) phase. Sometimes the cells maintain a quiescent phase without getting a proper signal for the subsequent division and enter to G0 phase, which is sometimes considered an extended G1 phase. Among many essential regulators, Cyclins and Cyclin-dependent kinases (CDKs) play a significant role in regulating the cell cycle. During cancer development, these checkpoints are dysregulated due to the dysfunction or hyperactivation of these proteins. In most cancers, the regulatory cascade behind the G1 to S phase is dysregulated and serves as a mitogenic signal for cancer development (Matthews, Bertoli and de Bruin 2022, 74-75).

C. Tumor suppressor genes and cancer

Tumor-suppressor genes code for the group of proteins that confine the cell division and promote programmed cell death hence alleviating the risk of tumor development (Lee and Muller 2010, 1-3). During oncogenesis, tumor suppressor genes are mutated, knocked down, or deactivated. The retinoblastoma (RB) gene encodes a nuclear protein, which mediates the cell cycle control checkpoint at the G1 phase, the first identified gene as a tumor suppressor. This gene mutation leads to the development of ovarian cancer or breast cancer. *p53*, one of the primary tumor suppressor genes, is a transcription factor that significantly contributes to growth arrest, DNA repair, and apoptosis (Mercadante and Kasi 2022). In 50%-70% of the total cancer population, the *p53* gene has been shown to be mutated (Rivlin, Brosh, Oren, and Rotter 2011, 466-470; Ozaki and Nakagawara 2011, 994-996). Another important Tumor suppressor gene, *BRCA2*, codes for DNA repair protein that further controls cell division, cell death, and repair of double-stranded DNA breaks. Mutation in this gene is significantly associated with breast cancer pathogenesis (Saleem, Ghazali,

Wahab, Yusoff, *et al.* 2020, 1-3; Mersch, Jackson, Park, Nebgen, *et al.* 2015, 269-272).

5. Cell metastasis, invasion, and angiogenesis in cancer

Metastasis (a term originally coined from the Greek methistanai that means moving to another new place), a key feature of malignancy, is defined as the capability to spread throughout the body and distinguishes cancer cells from normal somatic cells (Robert 2013, 333-336; Dudjak 1992, 40-44; Zeeshan and Mutahir 2017, 172-174). Cancer cells can penetrate beyond their primary site into lymphatic nodes and blood vessels, circulate through them, and invade the surrounding normal tissues elsewhere in the body to create another tumor mass. The overall process is regulated by a highly coordinated cascade of small events, sometimes termed the 'metastatic cascade (Eslami, Cortés-Hernández and Alix-Panabières 2020, 1-2).

Based on the sequence of events, the metastatic cascade can be divided into three major stages: Invasion, intravasation, and extravasation (Jiang, Sanders, Katoh, Ungefroren, *et al.* 2015, S245-S246) (Fig. 1-2).

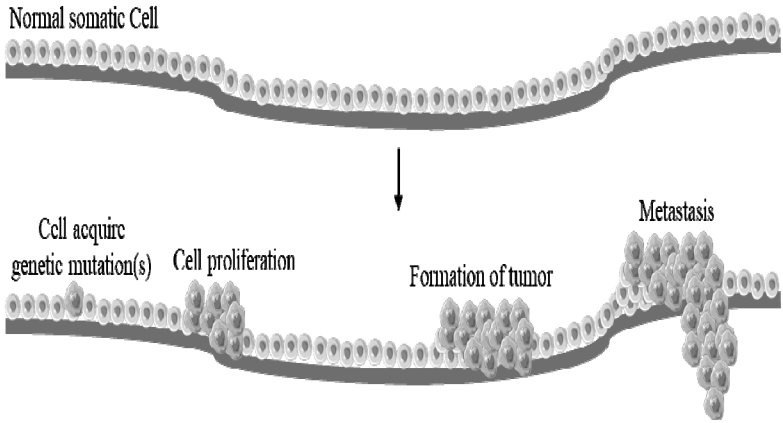

Fig.1-2. Stages of cancer development and progression

Invasion: The unique property of cancer cells defines invasion to dissociate from their origin or the primary tumor mass by the loss of cell-cell adhesion function and dysregulation in cell-matrix interaction. An invasion of the stroma of surrounding cells subsequently follows it. This process involves the following events:

A. Loss of cell-to-cell adhesion

The cell-to-cell adhesion mainly comprises tight junction (TJ), adherens junctions, gap junction, desmosomes, and integrin from apical to the basal membranes (Martin and Jiang 2009, 872-874). Dysregulation of any relevant structural proteins of these junctions can promote the loss of cell-cell association, inhibition of cell-to-cell contact, and degradation of the basement membrane. Among the number of TJ structural components, ZO-1, ZO-2, claudin-7, claudin-1, and occludin are directly involved in cancer (Martin, Mansel, and Jiang 2010, 723-725). HGF/SF (hepatocyte growth factor/scatter factor), stromal cell-derived cytokine, is also known to be significantly involved in metastasis in breast cancer development and progression (Utoguchi, Mizuguchi, Dantakean, Makimoto*, et al*. 1996, 24-34; Martin, Watkins, Mansel and Jiang 2004, 361-364). E-cadherin, a critical adherens junction molecule, gets mutated or downregulated in most epithelial cancers (Cavallaro and Christofori 2004, 121-123; Hsu, Meier, Nesbit, Hsu, *et al.* 2000, 1515-1518). Studies also have reported that desmosome-specific proteins, Desmoglein 2 and 3 and PKP3, are dysregulated in different types of cancers, including head and neck carcinoma, skin cancer, prostate and lung cancers (Furukawa, Daigo, Ishikawa, Kato, *et al*. 2005, 7102-7103; Breuninger, Reidenbach, Sauer, Ströbel*, et al*. 2010, 2509-2511; Brennan and Mahoney 2009, 148-150; Hsu, Meier, Nesbit, Hsu, *et al.* 2000, 1515-1518). Desmocollin 2, another essential desmosome protein, is reported to be associated with colorectal carcinomas (Kolegraff, Nava, Helms, Parkos, *et al*. 2011, 1121-1124). Besides, gap junction proteins Conexin43, Integrin, and Selectins are also reported to be associated with different types of cancer (Bendas and Borsig 2012, 1-3; Bonacquisti and Nguyen 2019, 439-442).

Once the cancer cells detach from their primary originated site, they invade the surrounding extracellular matrix, penetrating the blood vessels and entering the circulation. This event is termed intravasation (Martin, Ye, Sanders, Lane *et al.* 2013). After reaching another tissue, tumor cells eventually escape the blood circulation and build a tumor at a new site.

The overall process is called extravasation (Martin, Ye, Sanders, Lane *et al.* 2013).

B. Angiogenesis

During oncogenesis, cell proliferation, and metastasis lead to new growth of cancer mass. The newly formed cell mass requires a new vascular network to flow oxygen and nutrients continuously (Lugano, Ramachandran, and Dimberg 2020, 1745-1750). Angiogenesis defines the formation and development of new blood vessels (Nishida, Yano, Nishida, Kamura, *et al*. 2006, 213-216; Viallard and Larrivée 2017, 410-414). The formation of new lymphatic vessels is termed lymphangiogenesis (Nishida, Yano, Nishida, Kamura, *et al*. 2006, 213-216). Endogenous activators and inhibitors for angiogenesis are strongly associated with cancer development. The upregulation of angiogenic activators plays a significant role in the continuous demand for oxygen and nutrients, while negative regulators or angiogenesis inhibitors get downregulated. Among various activators of angiogenesis, VEGF family proteins (VEGF-A, VEGF-B, VEGF-C, and VEGF-E) play a significant role in cancer (Sullivan and Brekken 2010, 165-170). VEGF activates their corresponding receptors causing the proliferation of blood vessels (VEGF-A, VEGF-B) (Neufeld, Cohen, Gengrinovitch and Poltorak 1999, 9-14) and lymph nodes (VEGF-C and VEGF-D) (Rafii and Skobe 2003, 166-168; Mandriota, Jussila, Jeltsch, Compagni*, et al*. 2001, 672-678). Oxygen deprivation or hypoxia induces hypoxia-inducible factor-1α (HIF-1α), further increasing the expression of VEGF and its receptor (Bottaro and Liotta 2003, 593-595). Besides, some cytokines and growth factors also act as angiogenic activators. They might play a role in cancer development, including tumor necrosis factor (TNF)-α, transforming growth factor (TGF)-α and β, angiogenin, primary fibroblast growth factor (bFGF), platelet-derived endothelial growth factor, granulocyte colony-stimulating factor (G-CSF), placental growth factor, and epidermal growth factor (Nishida, Yano, Nishida, Kamura, *et al*. 2006, 213-219). Among angiogenic inhibitors, endostatin, thrombospondin angiostatin, interferon, and tissue inhibitor of metalloproteinase-1, -2, and -3 are known to play an important role (Nishida, Yano, Nishida, Kamura, *et al*. 2006, 213-219). This group of proteins gets downregulated in cancer, allowing them to serve as a potential therapeutic for cancer treatment (Nishida, Yano, Nishida, Kamura, *et al*. 2006, 213-219).