# Oncogenes: New Targets in Cancer Treatment

By

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Bachelor of Pharmacy (Hons.)

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## Declaration

It is hereby declared that

1. The thesis submitted is my own original work while completing degree at Brac University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I have acknowledged all main sources of help.

**Student's Full Name & Signature:**

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# Approval

The thesis/project titled Oncogenes: New Targets in Cancer Treatment submitted by Waseka Shams Chowdhury (15146052) of Spring, 2015 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy (Hons.) on 3rd October, 2019.

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## Ethics Statement

The study does not involve any kind of animal trial or human trial.

## Abstract

In the recent years, progression in cancer genetics and biology has shifted the paradigm for cancer drug development and design. Greater emphasis is given for the development of a specific, non-toxic and molecule based therapy to combat cancer. Therapies are designed to target the genes involved with growth, proliferation and metastasis of cancer. Oncogene based therapies have shown promising clinical activities such as imatinib, trastuzumab and gafitinib. However, there are obstacles to overcome to recognize the full potential of oncogenes as novel targets for cancer therapy. These include validating novel targets, designing of specific agents, and evaluating these agents in both clinical and preclinical settings, etc. It is also vital to have a strong understanding of the underlying molecular abnormalities and its mechanics to maximize the benefits of the molecular therapeutics in cancer.



**Keywords:** cancer, genes, proliferation, metastasis, oncogenes.

# Dedication

*Dedicated to the innocent lives taken away by cancer*

## Acknowledgement

First and foremost, all praises to the Almighty, for blessing me with patience, courage and the wisdom to follow my dreams while maintaining morale. Without His guidance it would be impossible to come this far.

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## List of Acronyms

| | |
|---|---|
| APUD | Amine Precursor Uptake and Decarboxylation |
| HIF | Hypoxia-inducible Factor |
| VHL | Von Hippel-Lindau |
| MAPK | Mitogen-activated Protein Kinase |
| PI3K | Phosphoinositide 3-kinase |
| FIH | Factor Inhibiting HIF |
| VEGF-A | Vascular Endothelial Growth Factor-A |
| PTEN | Phosphatidylinositol 3,4,5-triphosphate 3-phosphatase |
| ATP | Adenosine Triphosphate |
| hTERT | Human Telomerase Reverse Transcriptase |
| ECM | Extracellular Matrix |
| TGF | Transforming Growth Factor |
| TP53 | Tumor Protein 53 |
| NGF | Nerve Growth Factor |
| PDGF | Platelet-derived Growth Factor |
| MMTV | Mouse Mammary Tumor Virus |
| SCF | Stem cell factor |
| GTP | Guanosine-5‘-triphosphate |
| DLCL | Diffuse Large Cell Lymphoma |

| | |
|---|---|
| TRK | Tropomyosin Receptor Kinase A |
| GIST | Gastrointestinal Stromal Tumor |
| MGMT | Methyl Guanine Methyl Transferase |
| ADC | Antibody-drug Conjugate |
| CD | Cluster of Differentiation |
| HER2 | Human Epidermal Growth Factor Receptor 2 |
| PD-L1 | Programmed Death-ligand |
| TAA | Tumor Associated Antigens |
| GBM | Glioblastoma Multiforme |
| CRISPR | Clustered Regularly Interspaced Palindromic Repeats |
| DNA | Deoxyribonucleic Acid |
| RNA | Ribonucleic Acid |
| sgRNA | Single-guide Ribonucleic Acid |
| ENCODE | Encyclopedia of DNA Elements |
| EGFR | Epidermal Growth Factor Receptor |
| PDGFR | Platelet-derived Growth Factor Receptor |
| SCF | Stem-cell Factor Receptor |
| ALK | Anaplastic Lymphoma Kinase |
| FGFR | Fibroblast Growth Factor Receptor |
| Itk | Interleukin-2 Receptor Inducible T-cell Kinase |

| | |
|---|---|
| BRCA | Breast Cancer Gene |
| CDK | Cyclin-dependent Kinase |
| CDK7 | Cyclin-dependent Kinase 7 |
| CDK9 | Cyclin-dependent Kinase 9 |
| PARP | Poly (ADP-ribose) Polymerase |
| BRD4 | Bromodomain-containing 4 |
| PLK1 | Polo-like Kinase 1 |
| BTK | Bruton‘s Tyrosine Kinase |

# Chapter 1

# Introduction

## 1.1 What is Cancer

Cancer is common term used for characterizing a class of disease which refers to unmanageable growth of cells beyond their boundaries. Cancer can spread to surrounding tissues and invade organs. The term used to describe the invasive nature of cancer is malignancy. Tumors which show malignant characteristics are malignant tumors and neoplasms. Development of cancer may occur in almost any body part and has many sub types. Each sub type requires a separate management strategy.

Cancer has the second highest mortality rate globally and accounts for 9.6 million deaths in 2018, alone. Liver, stomach, colorectal, prostate and lungs are the organs where development of cancer is most common in men while thyroid, cervix, breast, colorectal and lung cancer is more prevalent in women (World Health Organization, 2019)

## 1.2 Evolution of Cancer

The incidence of cancer development steadily increases with the progression of a person‘s life with a gradual increase on occurrence from middle age. In the past few years, the genetic causes which are frequently associated with cancer development have been identified through traditional molecular genetic methods or through latest techniques such as next-generation sequencing.

Recently, applications mathematical models such as directed acyclic graphs and oncogenic trees has led to advancements in the analysis of cancer progression. Through the sequencing of the cancer genome, a more concise and direct study of the evolution of the tumors can be

obtained. Moreover, from the genomic sequencing of biopsies, algorithms are developed to reconstruct the historical evolution of the tumors and to pin-point the timing of the certain mutation and predict the sequence of the mutations (Ben-David, Beroukhim, & Golub, 2019)

To understand the progression and development of cancer and tumor growth, it is essential to decipher the mechanism of cancer growth which consists of a series of events. This provides better insight for the identification of the early steps in the evolution of tumors. Also, it opens the door for faster and better diagnostics, treatment, and prediction of cancer progression. The insights gained from the early studies on oncogenes led to the better understanding of how cancer develops. It was also deduced that oncogenes have a critical role on the promotion of gene instability. A timeline illustrating the key concepts and findings related to DNA damage and its impact on cancer development is provided (Fig. 1).

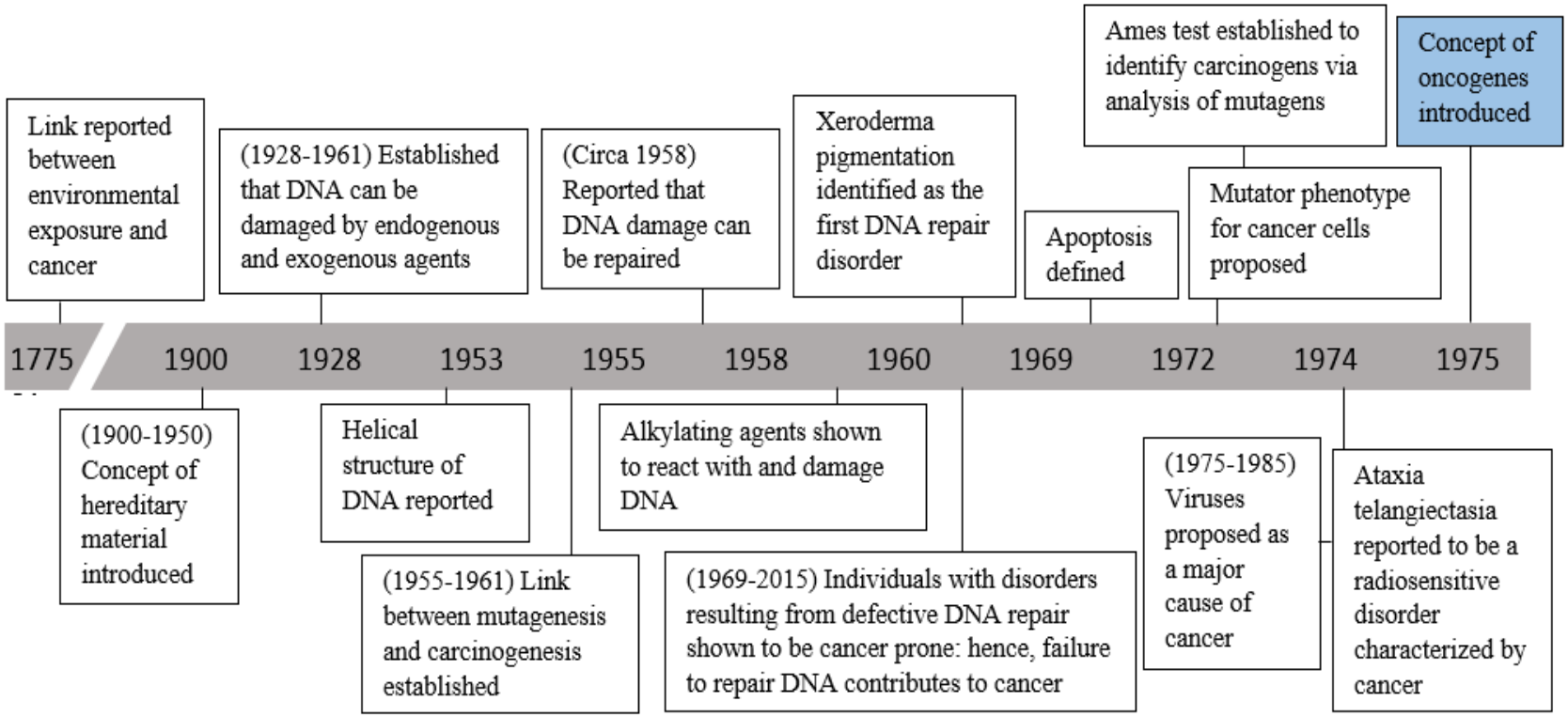


*Figure 1a: Timeline showing the development of cancer (1775-1975)*

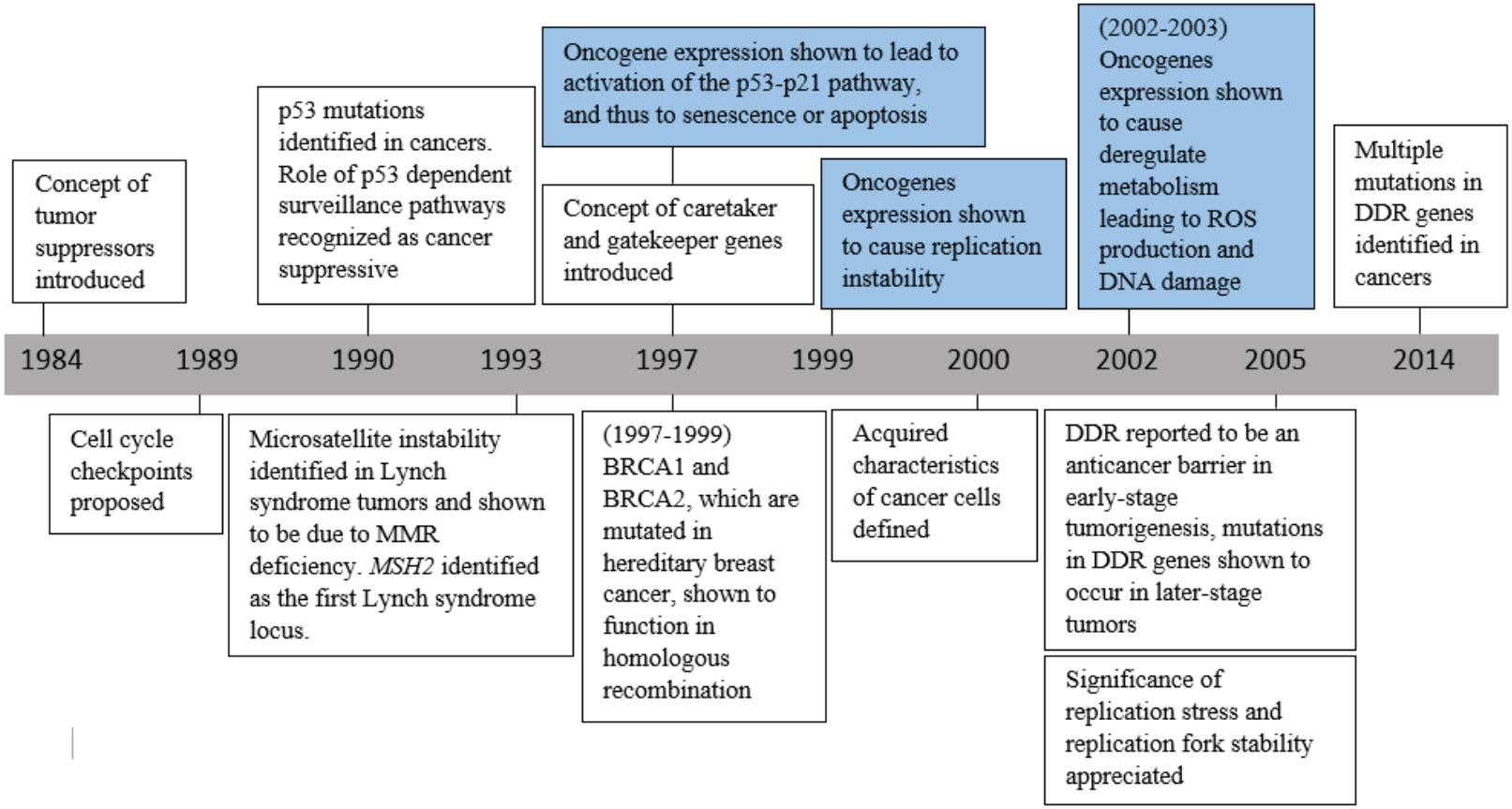


*Figure 1b: Timeline showing the development of cancer (1984-2014)*

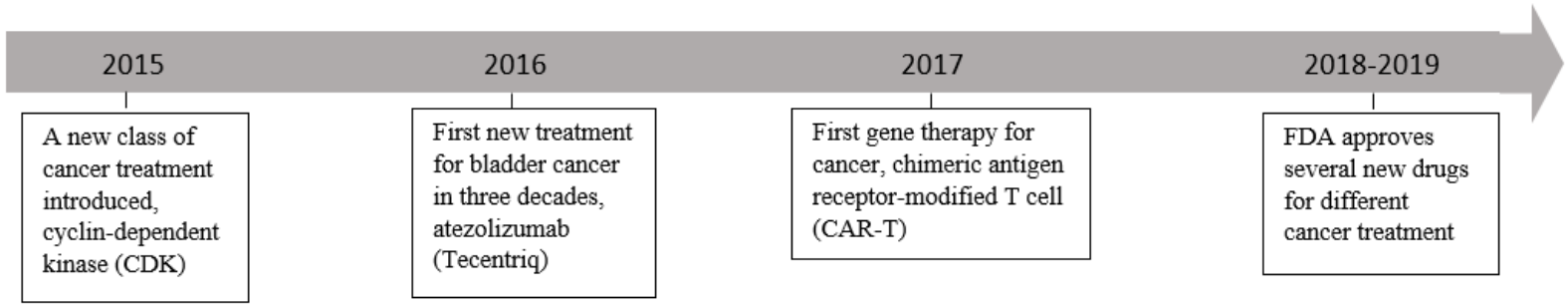


*Figure 1c: Timeline showing the development of cancer (2015-2019)*

## 1.3 Classification of Cancer

Tumors are mainly classified in two ways/ or as two types: benign and malignant. Tumors which do not spread to surrounding tissues and spread throughout the body are known as benign tumors, whereas tumors which spread and invade to surrounding tissues are known as malignant tumors.

These tumors are then classified on the basis of the tissue they are originated from. Can be categorized in three main types:

- Carcinomas,
- Sarcomas, and
- Leukemia or lymphomas.

More or less 90% of all cancers developed in human are categorized under carcinoma. Carcinomas are malignant cancers of the epithelial cells. It develops when the external or internal lining of the body‘s epithelial cells develop malignant characteristics. Epithelial tissues are located all throughout the body, found in the lining of the internal organs, their passage ways as well as in the skin. Carcinomas usually affects the organs and glands which are responsible for secretion. The breasts are responsible for the production of milk, or lungs, which are responsible for the secretion of mucus on the membranes. (National Cancer Institute, 2019).

There are two main subtypes of carcinoma: adenocarcinomas and squamous cell carcinomas. Adenocarcinomas develop in organs and glands. They usually generate in the mucus membranes as a thick white plaque-like mucosa. Adenocarcinomas spread throughout the soft tissues from their place of origin. On the other hand, squamous cell carcinomas originate in squamous epithelial cells and affect almost all parts of the body.

Sarcomas are another category of cancer, frequency of development in human is not very common. These are solid tumors of the muscles, fibrous tissues, cartilage, bones and other connective tissues (Cooper, 2000). Carcinomas usually affects the organs and glands which are responsible for secretion. Such as breasts, which are responsible for the production of milk, or lungs, which are responsible for the secretion of mucus on the membranes. (National Cancer Institute, 2019).

Examples of sarcomas are:

- Mesenchymous and mixed mesodermal tumor
- Chondrosarcoma
- Osteosarcoma and osteogenic sarcoma
- Rhabdomyosarcoma
- Fibrosarcoma
- Glioma or astrocytoma
- Myxosarcoma
- Leiomyosarcoma
- Angiosarcoma and hemangioendothelioma
- Liposarcoma
- Mesothelial sarcoma and mesothelioma

Approximately 8% of all human cancers are of the leukemias and lymphomas category. Leukemias originate from the cells which are responsible for the production of blood, while lymphomas originate from the cells belonging to the immune system.

According to the tissues from which tumors are developed such as lungs or skin, and also the sort of cell from which are they evolve, tumors are classified into different categories. For instance, cancer of the fibroblasts cause fibrosarcomas, and cancer of the erythrocytes cause erythroid leukemias (Cooper, 2000). Overproduction of the immature lymphocytes, or white

blood cells, is associated with leukemia. As these immature lymphocytes do not function properly the patient becomes prone to infections and other diseases. Erythrocytes, red blood cells, can also be affected by leukemia by causing formation of poor blood clots and development of anemia. The examples are as follows:

- Lymphatic leukemia
- Myelogenous and granulocytic leukemia
- Polycythemia vera and erythremia

The lymphatic system is composed of a scheme of nodes, organs and vessels which are responsible for the production of body fluids and lymphocytes. Occurance of lymphomas can be in the nodes or glands of lymphatic system. Lymphomas are also referred to as ‗solid cancers' and can affect specific organs like the brain, breast or stomach. This type of lymphomas is referred as extranodal lymphomas. Lymphomas can be further sub grouped into two types: Non-Hodgkin lymphoma and Hodgkin lymphoma. In Hodgkin lymphoma, Reed-Sternberg cells are present which is the distinguishing feature between these two types. (National Cancer Institute, 2019).

Different tumors arise from different body parts. The table below shows the different types of tumors which arise from the respective tissues.

- Tissues of the Connective System
- Tissues of the Mesothelium and Endothelium System
- Lymphoid and Blood Cells
- Muscle Tissues
- Tissues of the Epithelial System
- Tissues of the Neural System
- Tissues of the APUD System

- Other Tissues of the Neural Crest-Derived System

*Table 1: Tumors of the Connective System*

| Tissue | Benign Tumors | Malignant Tumors |
|---|---|---|
| Fat | Lipoma | Liposarcoma |
| Embryonic (myxomatous) fibrous tissue | Myxoma | Myxosarcoma |
| Cartilage | Chondroma | Chondrosarcoma |
| Bone | Osteoma | Osteosarcoma |
| Notochord | - | Chordoma |
| Adult fibrous tissue | Fibroma | Fibrosarcoma |
| Connective tissue, probably fibrous | Fibrous histiocytoma | Malignant fibrous histiocytoma |

*Table 2: Tumors of the Mesothelium and Endothelium System*

| Tissue | Benign Tumors | Malignant Tumors |
|---|---|---|
| Lymph vessels | Lymphangioma | Lymphangiosarcoma |
| Blood vessels | Hemangioma, hemangiopericytoma | Hemangiosarcoma, angiosarcoma |
| Mesothelium | - | Mesothelioma |

*Table 3: Tumors of Lymphoid and Blood Cells*

| Tissue | Benign Tumors | Malignant Tumors |
|---|---|---|
| Lymphoid tissue | Plasmacytosis | Plasmacytoma; multiple myeloma; Hodgkin lymphoma and Non-Hodgkin lymphoma |
| Hematopoietic cells | "Preleukemias", "myeloproliferative disorders" | Leukemia, of various types; aleukemic leukemia |

*Table 4: Tumors of Muscle Tissues*

| Tissue | Benign Tumors | Malignant Tumors |
|---|---|---|
| Striated muscle | Rhabdomyoma | Rhabdomyosarcoma |
| Smooth muscle | Leiomyoma | Leiomyosarcoma |

*Table 5: Tumors of the Epithelial System*

| Tissue | Benign Tumors | Malignant Tumors |
|---|---|---|
| Stratified squamous | Papilloma<br>Seborrheic keratosis and some skin adnexal tumors | Squamous cell carcinoma; epidermoid carcinoma and some malignant skin adnexal tumors |
| Transitional epithelium | Transitional cell papilloma | Transitional cell carcinoma |

| | | |
|---|---|---|
| Glandular epithelium<br>1. Liver<br>2. Kidney<br>3. Bile duct | Adenoma<br>Hepatic adenoma<br>Renal tubular adenoma<br>Bile duct adenoma | Adenocarcinoma<br>Hepatoma: hepatocellular<br>Renal cell carcinoma; hypernephroma<br>Cholangiocarcinoma |
| Placenta | Hydatidiform mole | Choriocarcinoma |

*Table 6: Tumors of the Neural Crest-Derived System*

| Tissue | Benign Tumors | Malignant Tumors |
|---|---|---|
| Glial cells (of several types) | - | Glioma grade I-III, anaplastic; glioblastoma multiforme (grade IV) |
| Meninges | Meningioma | Malignant meningioma |
| Nerve cells | -<br>-<br>Ganglioneuroma | Neuroblastoma<br>Medulloblastoma<br>- |
| Nerve sheath | Schwannoma, neurilemmoma<br>Neurofibroma | Malignant meningioma<br>Malignant schwannoma<br>Neurofibrosarcoma |

*Table 7: Tumors of the APUD System*

| Tissue | Benign Tumors | Malignant Tumors |
|---|---|---|

| | | |
|---|---|---|
| Pituitary | Basophilic adenoma<br>Eosinophilic adenoma<br>Chromophobe adenoma | -<br>-<br>- |
| Throid (C cells) | C Cell hyperplasia | Medullary carcinoma of thyroid |
| Parathyroid | Parathyroid adenoma | Parathyroid carcinoma |
| Bronchial lininh<br>(Kultschitzky cells) | - | Bronchial carcinoid; oat cell carcinoma |
| Stomach and intestines | Carcinoid | Maliganant carcinoid |
| Adrenalmedulla<br>Pheochromocytoma | Pheochromocytoma | Malignant Pheochromocytoma |
| Pancreas | Islet celladenoma; Insulinoma; gastrionoma | Islet cell carcinoma |
| Carotid body and chemo-receptor system | Chemodectoma; paraganglioma | Malignant chemodectoma; malignant paraganglioma |

Endocrine function is featured by a series of cells called the APUD (Amine Precursor Uptake and Decarboxylation) system. This means they release a number of small polypeptide or amine hormones. The hormones can be seen only under electronic microscope as dense membrane bounded granules located in the cytoplasm.

*Table 8: Tumors of Other Neural Crest-Derived Cells*

| Tissue | Benign Tumors | Malignant Tumors |
|---|---|---|

| | | |
|---|---|---|
| Schwann cells of peripheral nervous system | Schwannoma, or neurilemmoma | Malignant schwannoma |
| Pigment-producing cells in skin, eyes; and occasional other sites | Nevus | Melanoma |
| Merkel cells in squamous epithelium (unknown function) | - | Merkel cell neoplasm (similar to oat cell) |

## 1.4 Types of Tumors

Most tumors are not harmful to their host as they are small in size and rarely spread to other body parts. These are classified as benign tumors due to their close resemblance and characteristics to normal cells. Benign tumors can form in different parts of the body and some of the most common forms of benign tumor include Adenomas, Lipomas, and Myomas. The benign tumor cells are confined to the applicable tissues by surface interaction molecules most likely as normal cells

Factor that distinguishes benign tumors from malignant tumors is the fibrous capsule they remain enclosed in. The fibrous capsules make it easier for the benign tumors to be removed by surgery. On the other hand, the cells of the malignant tumors have some common protein characteristics with the cell type they arise from. Malignant cells also grow and divide much more rapidly than normal. The malignant cells spread and invade the surrounding tissues and reach the circulatory system of the body. Metastasis is the condition when the tumor cells spread from their origin and localize in other parts of the body causing secondary areas of

growth. Thus, the distinguishing factor of benign and malignant tumor is the ability of the malignant tumors to metastasize (Lodish et al., 2000).

The main distinction between malignant tumors and benign tumors in a nutshell are that malignant tumors, unlike the benign tumors can:

- Spread to surrounding tissue,
- Damage surrounding tissue, and
- Induce the development of other tumors

Malignant tumors can be lethal in nature. However, there are some types of tumors which develop very slowly that they do not show any complications in the individual‘s lifetime. Benign tumors also become malignant if they start grow in an uncontrollable rate. Cancer cells stop behaving like normal cells once they bypass the growth inhibiting mechanisms and start to proliferate uncontrollably. Another characteristic of cancer cells is that they do not adhere together. The cancer cells break apart from their origin and travel the bloodstream to other areas of the body to localize in new parts by metastasis (Institute for Quality and Efficiency in Health Care (IQWiG), 2019). When malignant tumors remain in their place of origin it is termed as ‗carcinoma in situ‘. When the tumors stop growing, it is said that the tumor has become dormant. Dormant tumors begin to form their own blood supply by the formation of blood vessels through a process called angiogenesis. The extra oxygen, hormones and glucose required for the sustenance of the tumor cells is supplied by the new blood vessels. Once a tumor has all the resources it needs it starts to invade its surrounding. This is known as invasive cancer (Institute for Quality and Efficiency in Health Care (IQWiG), 2019).

## 1.5 How Benign Tumors Become Malignant

Before a benign tumor becomes malignant, the benign tumor must develop blood vessels around it to be supplied with all the essential materials, such as oxygen, glucose and hormones, it needs to grow. For this transition, the tumor needs to adapt to certain conditions. This is how in situ carcinoma develops into malignant tumors by breaking free from their site of development and affecting other body parts to invade other tissues. This process can be enhanced by increased production of acid (Raghunand, Gatenby, Gillies, 2003). Hypoxia, a condition in which oxygen is in low levels, promotes the progression of in situ cancer to malignant tumors. In this process, the cells which are resistant to the decreased pH caused by extracellular acidosis and possess upregulated glycolysis characteristics survive and grow. So, it is suggested that the transition of in situ cancer to invasive cancer is closely linked to the microenvironment of the in situ cancer (Feitelson et al., 2015).

Hypoxia increases the resistance to treatment of cancer patients and also favors the progression of tumors. When tumor cells proliferate they move further away from the blood vessels which give them their source of oxygen and nutrients. This leads to the development of an environment where oxygen and nutrients are present in scarce levels. Hypoxia induces molecular responses in somatic and cancer cells that activates the key transcription factor hypoxia-inducible factor. Hypoxia-inducible factor is responsible for regulating a series of genes which have direct link to tumor cell survival and treatment resistance (Brahimi-Horn & Pouysségur, 2007).

Hypoxia-inducible factors, also called HIFs, are complex dimeric proteins that have an integral function to produce responses to low oxygen concentrations in the blood. HIF-1 is the primary gene that is involved in homeostatic processes. It is done by increasing the vascularization in the hypoxic areas where the tumors are located (Ziello, Jovin, & Huang, 2007). HIFs are usually upregulated in the development of cancer and during metastasis processes. There are several different types of HIFs and each form has a unique role for

maintaining tumor growth. Increased levels of HIF-1alpha has shown positive correlation with tumor progression. Increased levels of HIF-2alpha has also shown correlation with tumor progression. Upregulation of HIF-3alpha has been shown by various types of cancers. Over expression and increased activity of HIF-alpha in cancer can also cause the loss of tumor suppressor genes like Von Hippel– Lindau (VHL), oncogene activation, and an enhanced activity of signaling pathways of Phosphoinositide 3-kinase (PI3K) and Mitogen-activated protein kinase (MAPK) (Maynard & Ohh, 2007). Abnormal checkpoint control of the cell-cycle, increased activation of oncogenes and inhibitions of tumor-suppressor gene are considered to be primitive in the initiation of tumor progression (Moehler, Ho, Goldschmidt, & Barlogie, 2003). For the tumor to grow a local network of blood vessels need to be established which can supply nutrients and oxygen to the tumor cells.

Although, the tumor cells proliferate at a rate which is faster than the vasculature development. As a result, the tumor cells adapt themselves to survive in an environment with low oxygen concentration (Corbet & Feron, 2017). In histological examination, it has been seen that tumors have a central core of cells which are necrotic in nature that develop due to the decreased level of oxygen and glucose. Thus, resulting in cell death (Moeller, Richardson, & Dewhirst, 2007). These types of cells have the characteristic to being resistant to chemotherapy. They possess this resistance as the chemotherpauti drugs are not able to reach these areas due to lack of proper blood supply. Hypoxic tumors have also shown an extracellular environment which has a lower pH than their corresponding tissues. This acidic nature develops as an outcome of the modified metabolism of the tumor cells. The decrease in pH is caused by elevated production of lactic acid and carbonic acid through the glucose metabolism pathways (Nair & Shah, 2017).

Pathways which are regulated by hypoxia

The key transcription factor, hypoxia-inducible factor-1 alpha is induced by hypoxic conditions. It is controlled by a proline hydroxylase enzyme. The pathways what are regulated by hypoxia are listed below:

- pH regulation
- Apoptosis
- Tissue invasion and metastasis
- Genetic instability
- Immortalization
- Growth-factor signaling
- Glycolysis
- Angiogenesis

Tumor growth is promoted by most of the pathways induced by hypoxia. Hypoxia also induces apoptosis. A balance needs to be maintained among these pathways in order allow the growth of tumors in hypoxic condition. Drugs which are responsible for the inhibition of HIF-1 alpha expression can also antagonize the interaction of HIF-1α with CBP/p300 proteins or inhibits the downstream functions of vascular endothelial growth factor and other genes. Cyclooxygenase-2 enzymes have the potential to be used in tumor therapy (Pezzuto & Carico, 2019).

Hypoxia-inducible factor

Hypoxic conditions cause the hypoxia-inducible factors to activate. It is done by the inhibition of posttranslational hydroxylation of alpha subunit. The alpha subunit is responsible for stability, binding to elements which respond to hypoxia and heterodimerisation in target genes. The posttranslational hydroxylation by prolyl hydroxylase

domain proteins, oxygen-dependent oxygenases, and factor inhibiting HIF (FIH) cause destabilization and inactivation of HIF-alpha (Pezzuto & Carico, 2019).

Cellular responses mediated by HIF

Activated non-hydroxylated HIF alpha and beta factors targets around 1–2% of our genes. This leads either to the promotion or suppression of the genes with successive upregulation or downregulation of gene products expression, respectively. A wide class of genes which are take part in angiogenesis, pH regulation, cell death and survival, metabolism, adherence, migration, extracellular matrix modelling and metastasis (Maynard & Ohh, 2007).

Angiogenesis:

Angiopoietin-2 and VEGF-A, are genes that are expressed by HIF mediation. These genes allow tumors to grow in hypoxic conditions by forming a vascular network, this phenomenon is called angiogenesis. Thus, an environment rich in oxygen and nutrients is developed which aids the tumor to grow (Ferrara & Kerbel, 2005).

pH regulation:

A decrease in extracellular pH, acidosis, is caused as a result of the cancer cells having an affinity for producing lactic acid by cytoplasmic glucose metabolism pathways. This acidosis is brought about by an increase in the production of lactic acid and carbonic acid. The acidosis is also aggravated by limiting vasculature. It is evident through substantial data that hypoxia promotes the invasive nature of tumor cells (Sullivan & Graham, 2007). The loss of E-cadherin function is associated with HIF activation. E-cadherin is a part of the adherence proteins located in the surface of cells. It suppresses the invasion and metastasis of tumors.

On the other hand, a regulatory gene of epithelial-mesenchymal transition, TWIST-1, is activated in hypoxic conditions. Also, cells which have survived acidosis have an added advantage of growing in acidic conditions. This makes the cells more aggressive in nature and develop invasive characteristics (Boroughs & DeBerardinis, 2015).

Cell survival or death:

A series of events is initiated by hypoxia that causes the tumor cells to proliferate. If the hypoxic conditions become too severe then the tumor cells might go towards apoptosis. It has been proven that tumor development is affected by the levels of hypoxia. The correlation of FIH protein and HIF-1 alpha transcriptional activation domains have an impact on the determination of cell fate. The differential actions of the three HIF-alpha subunits can either promote cell death or cell proliferation. The genes; bnip3L (bnip3-like), bnip3 and Bcl-2/adenovirus EIB 19 kDa-interacting protein 3 are induced in hypoxic conditions (Pavlova & Thompson, 2016).

Metabolism:

The genes that are involved in the metabolism of glucose are also mediated by HIF. A great amount of genes take part in cell metabolism, especially those of glucose, are regulated by HIF. Cancer cells divert the metabolism of pyruvate from mitochondrial oxidative phosphorylation to the conversion of pyruvate to lactic acid in the cytoplasm (Brahimi-Horn & Pouysségur, 2007). However, the latter process may be simplified but it produces fewer moles of adenosine triphosphate (ATP) per glucose molecule. The reduction of ATP is compensated by an increased expression of HIF in enzymes and transporters of glucose in glycolytic pathways; this in turn increases the rate of glycolysis and uptake of glucose. Not only does HIF channel glucose molecules towards glycolysis by suppressing the respiration of mitochondria but it also regulates the ratio of isoforms of the cytochrome c oxidase

enzymes, a component of electron transport chain, by optimizing low levels of respiration. This strategy protects cells from oxidative damage in hypoxia and also is a more efficient process (Boroughs & DeBerardinis, 2015).

Proliferation:

Cell proliferation is an essential part of cancer progression and development. It is caused by alterations in expression and activities of the cell cycle proteins. Stimulation of cell growth is caused by the activation of a number of signal transduction pathways. Development of hypoxic conditions and fibrogenic response are parts of the early stages of tumor development (Feitelson et al., 2015). Growth factors which are known for promoting cell proliferation are induced by hypoxia. Proliferation initiates cell regeneration and migration after the damage caused by acute and chronic hypoxia. Growth factors, like platelet-derived growth factor and transforming growth factor-beta, are stimulated by HIF-1 alpha. Stimulation of extracellular growth factors, protein kinases such as p42/p44 mitogen-activated protein kinase are used to regulate proliferation. This has shown to be responsible for the phosphorylation of HIF-1 alpha and activation of HIF-1 target genes. This pathway is also responsible for the activation of HIF-2 alpha (Feitelson et al., 2015).

In hypoxia, activity of phosphatidylinositol 3-OH kinase (PI3K) is enhanced in cells. PI3K is regulates cell proliferation and apoptosis suppression. It is also one of the main downstream mediators of several pathways of tyrosine kinase signaling. Phosphoinositide phosphatase PTEN inhibits the PI3K pathways. HIF-1 responses are also activated in PTEN mutations. Cell growth and proliferation is regulated by PTEN. The main cause of the many human cancers, such as prostate cancer, endometrial tumors and glioblastoma is the deletion or mutation of PTEN. So it can be concluded that, the mutations of PTEN promotes tumor growth by synergistically enhancing HIF-mediated responses (Pezzuto & Carico, 2019).

Blood-clot formation:

Blood clot formation is induced by hypoxia. The monocytes which are cultured in hypoxia cause the upregulation of expression of the transcription factor early growth response-1 (EGR-1). This expresses the cell surface tissue factor expression which leads to the deposition of vascular fibrin and formation of blood clots. The upregulation of tissue factors in hypoxia is also induced by vascular smooth muscle cells and mononuclear phagocytes. Features of cancer include increased coagulation, pulmonary embolism and deep vein thrombosis. Blood clots also activate platelets for the production of angiogenic factors such as VEGF to promote clot revascularization, and also tumor vascularization. During reoxygenation the EGR-1 pathway is activated and causes acute hypoxia (Boroughs & DeBerardinis, 2015).

Drug-export mechanisms:

HIF-1 can cause the development of resistance to chemotherapy and radiotherapy treatments. This resistance is caused by the overexpression of the P-gp drug efflux pump (Boroughs & DeBerardinis, 2015). It has been seen that HIF is also a part of the repair mechanisms of double strand breakage and in the upregulation in the expression of telomerase and human telomerase reverse transcriptase (hTERT) enzymes. The increased expression of the hTERT enzyme is an identification of uncontrollable cell division in cancer development (Stewart & Weinberg, 2006).

## 1.6 Cancer Cells and Somatic Cells

Cells are the building blocks of our body that grow and divide to form newer cells as per the requirements of the body. In normal conditions, our cells die when they get worn out or damaged, and are substituted by new cells. This regular process continues unless there is some genetic change causing the DNA of the cells – which carries the instructions they need

– are damaged. These damaged or un-healthy cells look different and may also have different properties. Genes are segments of DNA which code for a specific proteins. Thus, each gene must have the correct instructions for making its protein.

Microscopic examinations can be used to differentiate cancer cells from somatic cells. Cancer cells are less differentiated than benign tumor cells and somatic cells. The malignant cells possess the characteristics of undergoing uncontrollable cell growth and proliferation. The malignant cells have fewer specialized functions, undergo many mitoses, possess a prominent nucleoli and have a high nucleus to cytoplasm ration. The tendency of tumor cells to invade the surrounding tissues is an indication of malignancy (Feitelson et al., 2015).

From the study of cancer cell biology it has been found that cancer cells have a wide range of differences to somatic cells. For instance, cancer cells are unstable and their genes are susceptible to duplication, deletion and rearrangement. This is the reason why their progeny can display unusual traits. Although, a tumor is said to be monoclonal in nature, it contains cells with a diverse range of characteristics (Martincorena & Campbell, 2015).

Cancerous cells also differ from somatic cells on the basis of looks and behavior. Somatic cell nucleus is about one-fifth in size of the entire cell. However, in cancer cells the nucleus of the cell occupies most of the cell‘s volume. Cancer cells may also lose the special characteristics of the cell from which they arise from. For example, if cancer cells develop from mucus-producing cells the cancer cells may lose the ability to produce mucus overall. Likewise, cancer cells which develop from epithelial cells lack the lack amount of keratin the original somatic cell possesses (Martincorena & Campbell, 2015).

The main distinguishing factors between cancer cells and somatic cells is that in the case of cancer cells have lost their restrains on growth. This means the cancer cells undergo continuous mitosis whereas the somatic cells very rarely undergo mitosis. Cancer cells also

show peculiar characteristics when they are grown in a medium; two examples are the cancer cells lack contact inhibition and they have reduced dependence on growth factors to sustain in the environment. In contrast to somatic cells, cancer cells do not coordinate with any other cells in their environment. They proliferate uncontrollably in the tissue media. The ability of the cancer cells to divide uncontrollably without any restrains on cell growth is one of the hallmarks of cancer (Martincorena & Campbell, 2015).

Cancer cells usually show abnormalities in their mechanism of regulating cell proliferation, survival and differentiation. Another distinguishing factor among cancer cells and somatic cultured cells is that somatic cells show the phenomenon of density-dependent inhibition. Somatic cells can grow and multiply until a finite cell density is reached. This is also dependent on the availability of growth factors in the medium. The somatic cells cease proliferation at the $G_0$ stage of cell cycle and become quiescent. In contrast, cancer cells do not follow this phenomenon of density-dependent inhibition. Instead of producing response to the growth signals that usually cause the somatic cells to stop proliferation and enter the $G_0$ stage, the cancer cells resume to grow in the media (Feitelson et al., 2015).

So it can be said that the compared to somatic cells, cancer cells have lowered requirement for growth factors in order to survive and proliferate. This characteristic of cancer cells contribute to its uncontrollable proliferating feature. Cancer cells, in some cases, have their own supply of growth factors which they produce that they use to proliferate. As cancer cells multiply in uncontrollable numbers, the growth factors produced by them are in endless supplies causing auto-stimulation of mitosis. Cancer cells have decreased dependence of extracellular growth factors as a result of abnormalities in their signaling pathways.

Cancer cells also lack the cell to cell and cell to matrix interactions which somatic cells possess. Unlike somatic cells, cancer cells do not adhere to one another, this is due to the

reduced cell surface adhesion molecule expression. E-cadherin, a cell surface adhesion protein, activity is lost in cancer cells. As a result, cancer cells tend to spread out and not stay in a cluster together like somatic cells do. This gives the cancer cells the ability to invade other tissues and metastasize. This is why most tumor cells are much more rounder in shape as they do not attach themselves to surrounding cells or extracellular matrix.

Cancer cells and somatic cells also differ on the basis of contact inhibition. Somatic cells tend to migrate across the culture dish, when in vitro, until they reach a neighboring cell and come in contact. The contact inhibits further migration of the somatic cells. In contrast, tumor cells do not follow the phenomenon of contact inhibition and continue to grow and proliferate even after they come in contact with a neighboring cell. This is how cancer cells form multi-layers when they are grown in vitro (Feitelson et al., 2015).

The primary physical barrier of tissues is the basal lamina. Cancer cells which start to metastasize break away from their tissue of origin and move past the physical barriers of basal lamina. Once this barrier is breached, the tumor cells can reach the bloodstream and spread throughout the body to form secondary tumors in other parts of the body. To overcome this physical barrier, tumor cells produce high levels of proteasome enzymes and cell-surface protein receptors which are specific for the cell surface of the basal lamina. Plasminogen activator, a protease, is also secreted by tumor cells which helps to break the peptide bonds between the plasminogen proteins in the basal lamina. The plasminogen then converts to plasmin. Increased protease activity helps the tumor cells to metastasize by helping them to digest and breach the basal lamina. When the tumor cells break out of the basal lamina they can reach the bloodstream and invade other tissues. In order for secondary tumors to form the tumor cell must first adhere to the endothelial lining of the capillary and migrate to other tissues (Boroughs & DeBerardinis, 2015)

# Chapter 2

# Cancer Initiation and Progression

Cancer evolves as a result of a series of evolutionary events which involves the mutational changes in the genes, that is when the genetic alterations interrupt with the regular orderly process of the cell. Each mutation results in a new phenotype which helps in the development of carcinogenesis.

## 2.1 Carcinogenesis

In somatic cells, cell proliferation is restrained by these three factors:

1. Extrinsic interactions. This is a series of growth signals, both positive and negative, which somatic cells exchange within themselves and the extracellular matrix. Combination of these signals help to maintain a stable growth of the cell population.
2. Senescence. Somatic cells can finitely replicate until certain length of telomere is reached after which cell death occurs.
3. Substrate availability. Cells must possess an adequate supply of substrate for them to maintain their normal cellular functions and also replicate. These substrates are supplied through the blood supply to the tissues. In hypoxic conditions, the substrate concentrations may become low and cell death will induced (Leong, Aktipis, & Maley, 2018).

### 2.1.1 From Initiation to Hyperplasia

Growth signals, both positive and negative, are used to regulate cell proliferation. Mutations in the oncogenes and tumor suppressor genes in the initiation stage cause the tumor cells to overcome the barrier to the cells to uncontrollable proliferation. These mutations are listed as one of the major hallmarks of cancer. The cells must also develop a resistance to apoptosis at the initiation stage. The result of these mutations is the development of hyperplastic cells (Leong et al., 2018).

### 2.1.2 Hyperplasia to Early Carcinoma In Situ

For the tumor cells to grow without any restrains even after the initiation stage, the cell must take more adaptive measures to bypass all possible mechanisms which keeps the cell cycles in check. Somatic cells can replicate only a finite number of times due to the consistent shortening of the telomere of the chromosomes. The next step is to overcome this restriction of senescence pathway by upregulation of the enzyme telomerase. The telomerase enzymes prevents further shortening of the telomere segment of chromosome after cell replication processes. This gives the cell the ability to replicate indefinitely. Following this step, the hyperplasic epithelia continues to grow within physical barrier, forming a carcinoma in situ (Leong et al., 2018).

### 2.1.3 Early to Intermediate Carcinoma In Situ

The transformation from the early carcinoma in situ stage to further steps requires the upregulation of glucose. Upregulation of glucose has been seen in the development of malignant phenotype in cancer cells. The increased conversion on glucose to lactic acid in the fermentation process causes intratumoral hypoxia and shifts in the glycolytic pathways (Leong et al., 2018).

### 2.1.4 Intermediate to Late Carcinoma In Situ

Upregulated of glucose causes the production of lactic acid. This lactic acid reduces the extracellular pH, inducing apoptosis through the activation of p53-dependent caspase enzymes (Leong et al., 2018). To reduce the extracellular pH, the Na/H exchangers are upregulated. Other than that mutations in the p53 or pro-apoptotic pathways also prevent the cells from undergoing apoptosis (Testa, Castelli, & Pelosi, 2017).

### 2.1.5 Invasive Cancer

The end result of these evolutionary changes is a collection of cells with the ability for upregulation of glycolysis and the attained function of surviving and proliferating in hypoxic conditions. This collection of cells have the selective advantage of surviving in acidic conditions and to sustain in low oxygen levels. This advantage also promotes the clonal expansion and invasive nature of the cells giving them their malignant phenotype (Raghunand et al., 2003). Although acidic conditions can promote tumor cell invasion, it is sufficient on its own to exist the basal lamina and move into the bloodstream. These cells must also develop a resistance to anoikis, a special type of apoptosis that is stimulated by the changes of cell—ECM interactions. The cells must also eventually develop the ability to proliferate and sustain n when positive growth factors are absent. This results in the development of invasive cancer cells, where the cells can invade into the bloodstream and spread throughout the body. Upregulated HIF-1 and other transactivated genes, like VEGF, promotes angiogenesis leading to the development of invasive cancer (Testa et al., 2017).

### 2.1.6 Metastasis

With the growth of a cancerous tumor, it may extend to other parts of the body through the bloodstream or lymphatic system. This process is termed as metastasis. During metastasis, cancer cells invade other tissues and develop secondary tumors.

After all the evolutionary changes, the pre-invasive cells develop the feature of aerobic glycolysis. In aerobic glycolysis the glycolysis process can be performed in the presence of oxygen. Aerobic glycolysis is also one of the hallmarks for metastasizing cancer cells. There are some important differences between the microenvironments of the primary tumor and the metastasizing tumor. For instance, the pre-invasive cancer are avascular, while the invasive and metastasized cancer have vasculature access. The continuous production of lactic acid from glucose in aerobic glycolysis process is an important hallmark for metastatic characteristics (Testa et al., 2017).

For the tumor cells to start spreading and metastasizing, the cells must increase their invasive nature and enhance their extracellular environment to support the migration of cancer cells to invade surrounding cells. HIF-1 potentiates the migration and invasive nature of cancer cells by negative regulation of E- cadherin, autocrine motility factor, TGF-a, matrix metalloproteinase-2 enzyme, urokinase-type plasminogen activator receptor, lysyl oxidase enzyme and chemokine receptor 4 (Maman & Witz, 2018).

TWIST1 is a gene that codes for BHLH Transcription factor 1. The TWIST1 gene codes from the snail homolog 1 and 2, SNAI1 and the SNAI2 respectively. These two proteins cause the suppression of the expression of E-cadherin. E-cadherin is a molecule which is responsible for intercellular adhesion. The loss of E-cadherin activity in cancer cells allow the cells to metastasize and invade surrounding tissues. E-cadherin activity is not present in carcinoma cells. The expression of E-cadherin is downregulated due to transcriptional repression and is lost with the transition of b-cell adenoma to b-cell carcinoma, suggesting this is a hallmark for benign tumors only (Testa et al., 2017).

The barriers that restrict uncontrollable proliferation must be overcomes in order for cancer cells to grow and carcinogenesis to develop. Hanahan and Weinberg (Hanahan & Weinberg,

2011) have proposed a set of six hallmarks which are present in almost cancers. These hallmarks are essential for the sustenance of tumor growth.

- Evasion of apoptosis,
- Self-sufficient growth signals,
- Invasion and metastasis,
- Uncontrollable proliferation,
- Anti-growth signals insensitivity, and
- Sustenance of angiogenesis

Gatenby and Gillies (Gatenby & Gillies, 2008) extended these six hallmarks by proposing two more hallmarks to the list:

1. Evasion of anoikis.
2. Increased glucose consumption by enhanced glycolysis, and resistance to toxicity of decreased pH in tumor environment.

Getenby and Gillies have also put emphasis on the crucial role of the series of events which taks place in order to overcome the obstacles of the external environment of the tumor to allow carcinogenesis to develop. A map can be illustrated to show the impacts of the hallmarks on the progression of metastatic cancer from normal cells (Fig. 2). A prediction derived by this model is that acidosis and hypoxia are essential for the development of in situ carcinoma lesions and these adaptations are critical for the transition of in situ cancer to invasive cancer (Maman & Witz, 2018).

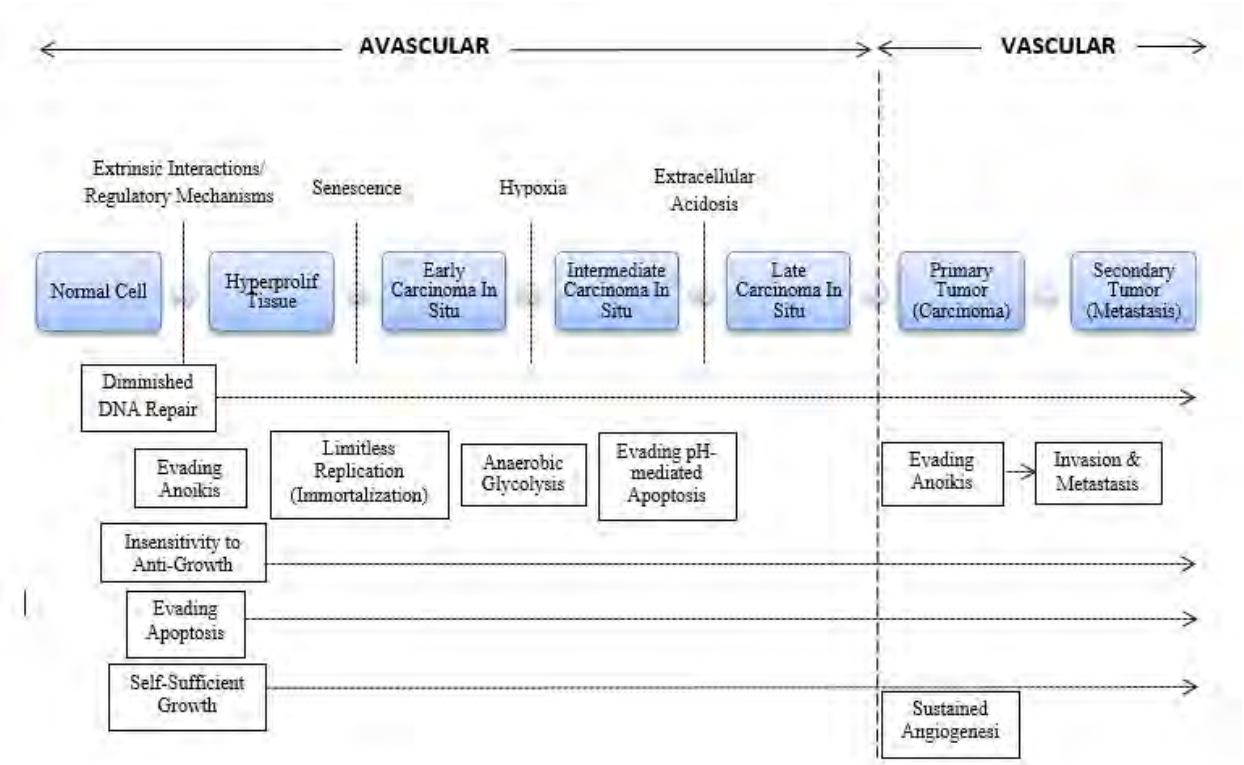


*Figure 2: A model illustrating the development of carcinogenesis, adapted from Gillies and Gatenby (Gatenby & Gillies, 2008).*

## 2.2 Causes of Cancer

For a cell to become cancerous, it must be genetically mutated and lose its regulation of growth factors. A tumor develops as a consequence of the uncontrollable proliferation of cells. This tumor may or may not be cancerous. If it is not cancerous it is termed as benign. Benign tumors do not spread to surrounding tissues. There are categories of cancer which do not produce any type of tumors. Examples of these type of cancers are; leukemia, lymphomas and myelomas. There are two types of genes which get mutated in a cell before the cell turns

cancerous; the oncogenes, this works as a positive growth regulator, another is the tumor suppressor gene, this works as a negative growth regulator.

The function of oncogene is to active the transmission of growth signals to the nucleus of the cell. Once this signaling pathway is disturbed by genetic alterations, cancer may develop. Likewise, mutation of the negative growth regulating tumor suppressor genes causes it to lose its function wither by inactivating it or by deleting its functional gene. The combined mutation of these two genes result in the progression of cancer (Blackadar, 2016).

Cancer is a disease that develops slowly with the progression of time. The development of cancer is a complex method with involves a succession of changes taking place in the genetic level. Each modification allows the precancerous cells to gain malignancy.

The two most common types of genetic mutations are as follows:

Acquired mutation: Acquired mutations are most prevalent. Damage in specific genes cause the development of cancer. Examples of cancer which occur through acquired mutations are; breast cancer and colon cancer. These type of cancers are termed as sporadic cancer. These type of mutations are not genetically inherited.

Germline mutations: These type of mutations are less prevalent than acquired mutations. These type of mutations occur in the reproductive cells. It can be genetically inherited from a parent. As the embryo grows, the mutation is amplified into all the cells. These type of cancers are termed as inherited cancer and it has a prevalence of 5-20%.

The two types of genes that show an important role in the progression of cancer are: proto-oncogenes and tumor suppressor genes. Proto-oncogenes, in their normal state, encourage cell division and growth. Whereas, tumor suppressor genes inhibits growth and cell division. By working in harmony, these two genes regulate the growth of cells and tissues and maintain homeostasis.

Oncogenes are the products of mutated proto-oncogenes. Oncogenes cause excessive cellular division. Mutations in the tumor suppressor genes inactive it. Thus, eliminating its function to inhibit cellular division. These two mutations combined, causes the cells to replicate in an uncontrollable manner eventually developing into cancer (Blackadar, 2016).

Cancer cells look for ways to escape the regulatory mechanisms which keep cell division in check. To do so, specific sets of genes which are responsible for the regulation of cell division such as, transcription factors, oncogenes, tumor suppressor genes, cell cycle inhibitors, enzymes and so on are manipulated. Oncogenes get activated by the following ways:

- Amplification in copy number;
- Hypo-methylation in the transcriptional control region;
- Point mutation;
- Gene translocation; or
- Insertion of provirus.

### 2.2.1 The Role of Oncogenes

Proto-oncogenes produce growth factors which stick to the receptors of specific cell for stimulating cell division. Oncogenes, mutated proto-oncogenes, cause overproduction of growth factors causing the cells to proliferate much faster than usual (Blackadar, 2016).

### 2.2.2 The Role of Tumor Suppressor Genes

Mutations in the tumor suppressor genes cause it to lose its inhibitory mechanism that counterbalance the growth stimulating signals. Cells lose one of its cell division checkpoints thus causing it to proliferate uncontrollably (Blackadar, 2016).

### 2.2.3 The Role of p53

Cells undergo programmed cell death, apoptosis, when the essential functions of the cell become compromised or damaged. Cancer cells avoid apoptosis by inhibiting the action of a tumor suppressor gene, p53 (Blackadar, 2016).

### 2.2.4 The Role of Telomerase

Each time a cell replicates a segment of its chromosome, called the telomere, shortens. Once the telomeres reach their threshold length the cells stop replicating. Cancer calls acquire an enzyme called telomerase which replaces the telomeric segments of chromosomes thus evading apoptosis (Blackadar, 2016).

### 2.2.5 The Role of TP53

The TP53 gene codes for the production of p53. This protein is a type of tumor suppressor; this means that it is responsible for the regulation cell division by preventing the cells from growing and proliferating uncontrollably. TP53 is transcription factor which is responsible for anti-proliferative responses of cell when they are under stress conditions. This gene prevents uncontrollable cell proliferation. Min cancer, mutations of TP53 inactivates the checkpoints for controlling cell proliferation (Blackadar, 2016).

### 2.2.6 The Role of Heredity

Some individuals may be more susceptible to the development of cancer than others due to hereditary factors. These factors may be lower production of melanin making the person rone to skin cancer, or any genetic mutations which promote uncontrollable cell growth (Blackadar, 2016).

## 2.3 Cancer Progression

A better comprehension of the series of events that lead to the progression of cancer would allow us to have a better prediction and provide earlier diagnosis to the patients. Still, the pathways leading to tumor progressions have not been characterized comprehensively. Now, with the advent of the whole genome sequencing methods, it is possible to infer the historical evolution of tumors from their genomes taken at biopsies and provide more insight for understanding tumorigenesis (Jolly & Van Loo, 2018).

Cellular genes are involved in cancer progression (Wheeler & Wang, 2013). The common genes that take part in cancer development have crucial implications in the understanding of the mechanism of cancer progression (Maman & Witz, 2018).

Almost all the genes take part in cancer development can be classified into the following two categories:

Tumor suppressor genes

Tumor suppressor genes are described as protective genes which keep the division and growth of cells within limit by:

- Restoring mismatched DNA
- Controlling the rate of cells division into new cells
- Controlling time of cell death

Cells grow uncontrollable when mutations occur in the tumor suppressor genes eventually leading to the formation of tumors. A few examples of tumor suppressor genes are: TP53 or p53 and BRCA1, BRCA2.

Oncogenes

When proto-oncogenes mutate they turn into oncogenes. Oncogenes stimulate excessive cell division turning somatic cells into cancerous cells. The two most prevalent types of oncogenes are:

- HER2, a protein which monitors cancer cell invasion and growth. HER2 genes are found in cancers like breast cancer cells and ovarian cancer cells.
- The RAS genes are accountable for the production of proteins that are responsible for cell communication pathways, cell growth and cell death (―The Genetics of Cancer | Cancer.Net," n.d.).

# Chapter 3

# Oncogenes

Oncogenes can be categorized into five different subtypes on the basis of the biochemical and functional properties. They are:

- Growth factors
- Growth factor receptors
- Signal transducers
- Transcription factors
- Programmed cell death regulators (Cisowski & Bergo, 2017).

Growth factor

Growth factors area type of polypeptides which are secreted to stimulate the proliferation of target cells (Hanahan & Weinberg, 2011). The target cells must possess the specific receptor to which the growth factors will bind to exhibit their action. An example of growth factor is the platelet-derived growth factor (PDGF); this is a protein of two polypeptide chains (Betsholtz, Karlsson, Lindahl, 2001). During blood coagulation, platelets release PDGF. It induces fibroblast proliferation which is responsible for wound healing. Nerve growth factor (NGF), fibroblast growth factor and epidermal growth factor are examples of growth factors.

The relation among retroviral oncogenes and growth factors was discovered after the study of sis oncogenes in simian sarcoma virus. This is a type of retrovirus found in fibrosarcoma of monkeys. The sequence analysis of revealed that the sis oncogene encodes for the beta chain of PDGF. It was concluded from this discovery that the inappropriate expression of growth factors could function as oncogenes. It was observed from experimental studies that PDGFb constitutive expression, a sis gene product, was adequate for neoplastic transition of

fibroblasts. But this did not happen in cells where PDGF receptors (PDGFR) was absent. Autocrine stimulation is when growth factors affect the cells which it is produced from. Neoplastic transformation is caused by autocrine stimulation of the sis gene product. This results in cell proliferation. The Int-2 gene is a growth factor which can act as an oncogene. It is a part of the fibroblast growth factor family which is stimulated in the mammary carcinomas of mice by MMTV insertional mutagenesis (Cisowski & Bergo, 2017).

Growth factor receptors

Viral oncogenes are mutated forms of growth factor receptors. They possess intrinsic tyrosine kinase activity (Cisowski & Bergo, 2017). The tyrosine kinase receptors have a unique structure of three domains:

- Transmembrane domain
- Extracellular ligand-binding domain, and
- Intracellular tyrosine kinase catalytic domain.

Information is transmitted unidirectionally in growth factor receptors through cell membrane. The intracellular tyrosine kinase catalytic domain gets activated by the attachment of the growth factor to the receptor‘s extracellular ligand-binding domain. Specific proteins are phosphorylated and recruited through the activated receptor to initiate a sequence of events which eventually leads to division of cells.

Examples of growth factor receptors include; trk, ros, ret, met, kit, fms, erb B1 and erb B2. Mutations of these growth factor receptors can lead them to oncogenes (Miranda et al., 2002). For example, the removal of the ligand-binding domain of erb B results in the constitutive activation of the receptor even in the absence of the ligand binding domain. Point mutation in the extracellular domain or the tyrosine kinase domain and removal of the intracellular regulatory domains can also cause the constitutive activation of receptor tyrosine kinases.

Examples are kit, erb B1 and erb B2. Sequencing of the erb B2 gene has revealed that it is found in 5% of gastric carcinomas, 4% of breast cancers and 3% of colorectal cancers.

Sequencing of the erb B1 gene revealed that this mutations were more prevalent in non-small-cell lung cancer and colorectal cancer. It has been also found that erb B1 mutations are more prevalent in female patients who have never smoked (Cisowski & Bergo, 2017).

Tyrosine kinase glycoproteins are encoded by the kit proto-oncogene. The binding of kit and its cognate ligand stem cell factor (SCF) results in receptor homodimerization, phosphorylation of Akt and STAT3 and kit tyrosine kinase activity activation. The three general mechanisms by which kit is activated in tumor cells are described below:

- Kinase cross-activation and/or loss of regulatory phosphatase activity
- Pancreatic and/or autocrine receptor stimulation by its ligand, SCF; and
- Activation of mutations of several different exons of the kit gene.

Kit mutations typically takes place in exon 17. It is mostly commonly found in myelogenous leukemia (AML), mast cell leukemia, sinosal natural killer/T-cell lymphoma ad acute seminoma/dysgerminoma. Increased or unusual growth factor receptor expression can also cause neoplasia. The experimental study of mutated growth factor receptors in neoplasia have helped us to understand how cell proliferating mechanisms are regulated.

Signal transducers

Mitotic signals are communicated from growth factor receptors from the cell membrane to the cell‘s nucleus across a sequence of complicated connecting pathways which is together referred to as the signal transduction cascade (Pollock & Meltzer, 2002). This broadcast of information is attained by the sequential phosphorylation of the cytosil proteins. Guanine

nucleotide-binding proteins and second messengers are also involved in signal transduction. The first retroviral oncogene discovered to be involved in signal transduction pathway is src.

Many proto-oncogenes are also components of the signal transduction pathways. They belong to two main groups; the guanosine triphosphate (GTP) binding proteins and non-receptor protein kinases. Proteins which bind to GTP possess intrinsic guanosine triphosphatase (GTPase) enzyme activity. These are further divided into monomeric and heterotrimeric groups. Monomeric GTP-binding proteins plays a vital role on ras family of proto-oncogenes which comprise of H-ras, K-ras, and N-ras. Heterotrimeric GTP- binding proteins implicated as proto-oncogenes include gsp and gip.

The non-receptor protein kinases can be further subclassified into serine/ threonine kinases, pim-1, mos and raf-1, and tyrosine kinases, src, lck and abl. Signal transducers are usually transform into oncogenes by mutations thus leading to unchecked activity and uncontrollable cellular proliferation (Cisowski & Bergo, 2017).

Transcription factors

Transcription factors are a type of protein which take part in the regulation of targeted genes expression. Transcriptional regulation is controlled by the attachment of certain proteins to DNA sequences which are usually located upstream with respect to the target gene. Transcription factors are part of the multiple gene families which have a general DNA-binding domains like zinc fingers. Other proteins are a part of the mechanism of action of transcription factors. The ultimate relationship of the signal transduction pathway are the transcription factors which convert extracellular signals to control alterations in gene expression.

Transcription factors also function as proto-oncogenes were discovered through retroviral homologues. The examples are c-myc, myb, jun, fos, ets and erb A. The combination of jun

and fos forms the AP-1 transcription factor which is responsible for the positive regulation of target genes whose expression directs to cell division.

The receptor for triiodothyronine is Erb. Proto-oncogenes which works as transcription factors are usually stimulated by the chromosomal translocations in solid and hematologic neoplasms. In some types of sarcomas, chromosomal translocation causes the generation of fusion proteins which are involved in the connection of EWS gene with other genes this results in transcriptional activation of tumor-associated. The c-myc gene is an important example of a proto-oncogene in human hematologic tumors which also have transcriptional activity. This gene helps to control the expression of genes which are responsible for cell proliferation (Cisowski & Bergo, 2017).

Programmed cell death regulation

A balance is maintained among cellular proliferation and cellular death in normal tissues. Programmed cell death is an essential part in the process of organ development and embryogenesis. Apoptosis is a form of programmed cell death which takes place in mature tissues (Goldar, Khaniani, Derakhshan, & Baradaran, 2015). This process involves the bulging the plasma membrane, nucleus condensation, volume contraction, and cleavage formation of the DNA by endogenous nuclease enzymes into fragments of nucleosome-sized. Apoptosis is induced in mature cells by external stimuli such as radiation exposure or steroids. The combination of uncontrollable regulation of cell proliferation and failed system of programmed cell death leads to the development of cancer.

Bcl-2 is the first proto-oncogene to have to regulate programmed cell death. It was concluded from experimental studies that the activation of bcl-2 inhibits programmed cell death. The main mechanism of action of activating bcl-2 is classified as an oncogene. The bcl-2 gene codes for a protein which is situated in the inner mitochondrial membrane, nuclear membrane

and the endoplasmic reticulum. Studies suggest that the bcl-2 protein functions as an antioxidant which restrains lipid peroxidation of cell membranes. Also, bcl-2 homologues which attach to bcl-2 have been recognized. This suggests that bcl-2 has a role in protein-protein interactions. The site-directed mutagenesis of the two bcl-2 proteins BH1 and BH2 domains have demonstrated that these two portions are crucial for binding of bcl-2 to bax. Bax is associated with bcl-2-family which is responsible for promoting cell death. Bax interacts with bcl-2 to regulate the apoptotic pathway. Even though the mechanism of bcl-2 gene activation is translocation, bcl-2 point amplifications and also have been reported (Danial, 2007). Amplification of the bcl-2 gene have been reported in 30% cases of high-grade diffuse large cell lymphomas (DLCL).

Caspase-9 is another oncogene that has been proven to be involved in apoptosis. This oncogene is activated by the intrinsic pathway. The caspase adaptor pro-caspase-9 and Apaf-1 is activated by the release of cytochrome c into the cytosol. This produces a holoenzyme complex called apoptosome. Downstream caspases, caspase-3, 6, 7 and 8 are activated by caspase-9. These downstream caspases cause apoptosis and DNA fragmentation. Akt can also regulate apoptosome function by caspase-9 phosphorylation (Ichim & Tait, 2016). The table below summarizes the five major subtypes of oncogenes and their respective neoplasm (Table 9).

*Table 9: Oncogenes*

| Oncogenes | Neoplasm |
|---|---|
| Growth factors | |
| v-sis | Constitutive production |
| int2 | Constitutive production |
| KS3 | Constitutive production |
| HST | Constitutive production |

| | |
|---|---|
| Growth factor receptors | |
| *Tyrosine kinases: integral membrane proteins* | |
| EGFR | Gene amplification/protein/point mutation |
| v-fms | Constitutive activation |
| v-kit | Constitutive activation/ point mutation |
| v-ros | Constitutive activation |
| MET | DNA rearrangement/ ligand-independent constitutive activation (fusion proteins) |
| TRK | DNA rearrangement/ ligand-independent constitutive activation (fusion proteins) |
| NEU | Gene amplification/ point mutation |
| RET | DNA rearrangement/ point mutation |
| Signal transducers | |
| *Cytoplasmic tyrosine kinases* | |
| SRC | Constitutive activation |
| v-yes | Constitutive activation |
| v-fgr | Constitutive activation |
| v-fes | Constitutive activation |
| ABL | DNA rearrangement |
| *Membrane-associated G proteins* | |
| H-RAS | Point mutation |
| K-RAS | Point mutation |
| N-RAS | Point mutation |
| BRAF | Point mutation |
| Gsp | Point mutation |
| Gip | Point mutation |
| *GTPase exchange factors (GEF)* | |
| Dbl | DNA arrangement |
| Vav | DNA rearrangement |

| | |
|---|---|
| *Serine/threonine kinases: cytoplasmic* | |
| v-mos | Constitutive activation |
| v-raf | Constitutive activation |
| pim-1 | Constitutive activation |
| *Cytoplasmic regulators* | |
| v-crk | Constitutive tyrosine phosphorylation of cellular substrates (eg. Paxillin) |
| Transcription factors | |
| v-myc | Deregulated activity |
| N-MYC | Deregulated activity |
| L-MYC | Deregulated activity |
| v-myb | Deregulated activity |
| v-fos | Deregulated activity |
| v-jun | Deregulated activity |
| v-ski | Deregulated activity |
| v-rel | Deregulated activity |
| v-ets-1 | Deregulated activity |
| v-ets-2 | Deregulated activity |
| v-erbA1 | Deregulated activity |
| v-erbA2 | Deregulated activity |
| Others | |
| BCL2 | Constitutive activity |
| MDM2 | Gene amplification/ increased protein |

## 3.1 Mechanism of Oncogene Activation

Oncogenes are products of the mutations on proto-oncogenes. The result of this mutation is that the cells can now grow uncontrollably. There are four mechanisms by which oncogenes are activated:

- Mutation,
- Gene amplification, and

- Chromosome rearrangements.

Mutation, gene amplification and chromosome rearrangements are responsible for the increased expression or the structural alteration of proto-oncogenes. As cancer development is a multistage process, all of these mechanisms take part in the development of cancer by causing genetic mutations. For cancer cell to metastasize the proto-oncogenes need to be triggered and the tumor suppressor genes need to be inactivated. The combination of these two genes are essential for the development of cancer (Hnisz et al., 2016).

Mutation

Structural alterations are caused by mutations in the proto-oncogenes. When these mutations take place in the critical protein regulatory regions or the catalytic domains it leads to the uncontrollable activation of the proteins. The types of mutations which cause activate proto-oncogenes are; insertions, deletions and base substitutions (Hnisz et al., 2016). Deletion mutations in the amino terminal of the ligand binding domains activate retroviral oncogenes. Examples include removal of the ligand-binding amino-terminal domains of the erb B, met, and trk oncogenes (Todd & Wong, n.d.). The most common type of mutation caused in human tumors is base substitution. This changes one amino acid in the protein.

The most common type of mutation in proto-oncogene is in the ras family. The mutation occurs in the N-ras, N-ras and H-ras (Hnisz et al., 2016).

The signal transductions from the stabilized ras proteins initiate malignancy of tumors. Stabilization is caused by point mutations taking place in the codon 12 level region and other mutations in the codon 13 or 61 regions. It has also been proven that the mutations in the codon 146 regions of K-ras proteins can also lead to the progress of certain types of cancer, such as non-small-cell lung carcinoma. K-ras mutation also causes a number of other types of cancer, which include colorectal cancer and pancreatic cancer (Hnisz et al., 2016). This

observation was made after finding evidence of only K-ras, but no H-ras or N-ras, is responsible for promoting the expansion of endodermal stem cell and hinder its differentiation. Hematologic malignancies are caused by N-ras mutations, with an incidence of 25%. K-ras, N-ras and H-ras mutations cause cancer in the thyroid tissues (Graziano & Gonzalo, 2017).

Other than in the development of cancer, mutations in the ras family are also involved with other diseases. For example, human autoimmune lymphoproliferative syndrome is caused by mutations in the N-ras protein, (Hnisz et al., 2016). while K-ras and H-ras mutations in their germ line causes diseases of the Noonan syndrome spectrum.

Point mutations occurring in the BRAF gene is also involved in the progression of cancer. This was the first cancer gene to be identified in the human genome project. The BRAF gene is composed of three CR1 regions which are conserved. The CR1 region codes for the putative zinc finger domain, CR2. In this CR2 region there is a high number of threonine and serine. Finally, the CR3 region is known as the kinase domain. The BRAF gene binds to the ras-GTP complex and is transferred to the plasma membrane this causes signal transductions to be with the mitogen-activated protein (MAP) kinase pathway. BRAF gene mutation is the most basic type of oncogenic mutation which is incident in almost 90% of the cancer cases. This mutation is caused by the change in the valine amino acid at the $600^{th}$ position in the BRAF kinas domain. The valine residue is converted to glutamic acid. This mimics the serine 602 and threonine 599 phosphorylation process. These two regions in the BRAF gene are responsible for the activation of ERK protein (Graziano & Gonzalo, 2017). BRAF gene mutations were found with 14% incidence in ovarian cancer, 12% incidence in colorectal cancer, 45% incidence in throid cancer and 75% incidence in melanomas. BRAF mutations occur in all the cases of acute lymphoblastic leukemia. It has been found that the BRAF gene mutation is alone responsible for the development of colorectal cancer even when K-ras gene

mutations were not present, and also in papillary thyroid cancer where rearrangements of TRK and RET were not present. These observations have led to the conclusion that TRK, RET and BRAF alterations or K-ras and BRAF mutations have similar effect on the cancer development in thyroid and colorectal, respectively.

BRAF mutations are also present in serrated adenomas and hyperplastic polyps which suggests that they are responsible for the development of these type of lesions (Hnisz et al., 2016).

Point mutations in the ret proto-oncogene is involved with multiple endocrine neoplasia type 2 syndrome and other carcinoma like familial medullary thyroid carcinoma. Multiple endocrine thyroid 2A is caused by mutations taking place in codon 634. This mutation affects one of the few cysteine residues in the juxtamembrane domain location of the receptor. This leads to the development of oncogenic potential as a consequence of the stimulation of the tyrosine kinase activity of the ret receptor(Graziano & Gonzalo, 2017).

Gene Amplification

Gene amplification is known as the increase in the copy number of a gene. The concept of gene amplification was revealed when studying the mechanism of acquiring resistance of growth inhibiting drugs in tumor cell lines. Inessential replication of the genomic DNA leads to gene amplification. This gives rise to karyotypic abnormalities such as HSRs and souble-minute chromosomes (Bagci & Kurtgöz, 2015).

A double minute chromosome (DM) is a structure which lacks a centromere. HSRs are portions of the chromosome which lacks the usual alternating patterns of dark and light stained bands. A large number of DNA amplification is represented by DMs and HSRs. Amplifications in an area of the DNA leads to the increased gene expression. This gives a selective advantage to growth. Gene amplifications are evident in the many cases of neoplasia

It was later discovered through experimental studies that the amplification of three proto-oncogene families, ras, erb B and myc, led to development of tumors. C-myc amplification is seen in 20-30% cases of ovarian cancer, squamous cell carcinoma and breast cancers. N-myc amplifications were seen in tumor progression of advanced neuroblastomas. L-myc amplifications were see in bladder neoplasia, neuroendocrine-derived tumors and small-cell carcinoma of lungs (Bagci & Kurtgöz, 2015). C-myc amplifications and erb B2 alterations are associated in the transition of non-invasive to invasive for tumor progression in breast carcinomas. Erb B amplification incident was found in 50% cases of colorectal cancer, 10-20% cases of squamous carcinomas of the neck and head and 50% cases of glioblastoma. Erb B2 gene overexpression and amplification is also associated in the development of 25% cases of salivary gland, gastric, endometrial, ovarian and breast carcinomas. It was also incident in 16% cases of non-small-cell lung carcinoma and gastrinoma. The table below summarizes the specific oncogenes which are amplified in certain types of tumors (Table 10).

*Table 10: Amplified Oncogene in Human Cancer*

| Tumor Type | Amplified Gene |
|---|---|
| Neuroblastoma | MYCN |
| Glioblastoma | ERB B1 (EGFR) |
| Small-cell lung cancer | MYC |
| Breast cancer | MYC, ERB B2 (EGFR 2), FGR1, FGR2, CCND1 (cyclin d1) |
| Gastric cancer | K-RAS, CCNE (cyclin e) |
| Esophageal cancer | MYC, CCNDQ (cyclin d1) |
| Sarcoma | MDM2 |
| Hepatocellular cancer | CCND1 (cyclin d1) |
| Cervical cancer | CDK4, MYC |
| Ovarian cancer | MYC |
| Head and neck cancer | MYC |
| Colorectal cancer | ERB B2 (EGFR 2) |

Chromosomal Rearrangements

Persistent chromosomal rearrangements are mainly initiated in hematological malignancies and also in some cases of solid tumors (Bagci & Kurtgöz, 2015). These rearrangements mainly comprises of inversions and translocations of chromosomal. Hematological malignancies are caused by chromosomal rearrangements by two main mechanisms:

- Transcriptional activation of proto-oncogene
- Creation of fusion gene

Transcriptional activation, also known as gene activation, arises from rearrangements of the chromosome which brings a proto-oncogene closer to a T-cell receptor gene. The regulatory elements of the T-cell receptor locus then regulate the transcription of the proto-oncogene. This results in the deregulation of the proto-oncogene expression. This causes the cell to undergo neoplastic transformation.

When a chromosomal breakpoint falls in between the loci of two different genes a fusion gene is formed. The result is a juxtaposition segment of two different genes. This causes the formation of a composite structure which is composed of a tail of one gene and the head of another. Fusion genes code for proteins which are chimeric in nature with transforming activity. Generally, both the genes which take part in the formation of the fusion takes part in the chimeric oncogene‘s transforming potential. Mistakes in the T-cell receptor genes rearrangement gives rise to several of persistent chromosomal rearrangements in hematologic malignancy (Bagci & Kurtgöz, 2015). In many cases, several translocations are caused by the same proto-oncogene.

## 3.2 Oncogenes as Target of New Drugs

Oncogenes take part in the key points of the cell life. Most oncogenes act in the signal transduction pathways and code for growth factor receptors. As a result, they are used as targets for new drug development. These new drugs are targeted to block cell deregulation. Few of the new insights of targeted drug therapy are summarized below (Dang, Reddy, Shokat, & Soucek, 2017).

ERB B2

Amplification of the ERB B2 gene is incident in a great number of breast cancers. Trastuzumab is an antibody that is used against erb B2 receptors. This drug has entered clinical trials and has shown some promising results which have benefitted patients. Trastuzumab is used for metastatic breast cancer. It is alos under observation to be administered in other adjuvant therapies. However, transtuzumab is not effective in 100% patients having the amplified erb B2 gene. A possible explanation could be because of the deregulation of erb B2 gene in the downstream regulations. It has also been found that point mutations in the PIK3CA gene and absence of PTEN protein expression leads to resistance development for trastuzumab (Dang et al., 2017).

ERB B1

The ERB B1 has an important impact on angiogenesis, cancer cell proliferation and metastasis. It does so by coding for the tyrosine kinase receptor. This means by targeting the erb B1 gene cancer therapies can be developed. Two types of erb B1 antagonists have been successfully developed and tested in phase III trials. The two classes are: inhibitors of small molecule tyrosine kinase and monoclonal antibodies.

Pantitumumab and cetuximab are two types of monoclonal antibodies. These competitively attach to the inactive receptor‘s extracellular domains by occluding the ligand-ligand region, and therefore blocking the receptor‘s ligand-induced tyrosine kinase

10-20% non-small-cell lung cancer patients partially respond to the treatment with gefitinib or erlotinib. Studies have confirmed that patients with EGFR mutation are particularly more sensitive to gefitinib or erlotinib treatments they have a response rate of 80% in patients. The erb B1 gene has a number of reported, but only four drugs have been validated so far. Additionally, the copy number gain of the erb B1 gene has been reported as another method for patient selection (Dang et al., 2017).

Advanced colorectal cancer has been treated with monoclonal antibodies. Apnitumumab and cetuximab monoclonal therapies have a response rate of about 9-12% which has to upto 20% with the use of irinotecan in combination with the monoclonal antibody therapies. However, for an advanced colorectal cancer patient to use this treatment he/she should possess evidence of overexpression og EGFR in the primary tumors. However, this methodology does not represent the gold standard to evaluate erb B1 alterations. Cetuximab and panitumumab are also effective in 10-13% cases of patients who are affected by squamous cell carcinoma of the head and neck (Dang et al., 2017).

KIT-pdgfra

Recently, it has been seen that imatinib can be used in the treatment of GIST by acting as a tyrosine kinase activity inhibitor in bcr-abl-positive leukemia. 85-90% response can be seen patients with mutations in exon 11, while upto 50% response can be seen in patients with mutation in exon 9. From these results, it can be concluded that all patients of GIST metastasis can receive imatinib treatment (Sanchez-Hidalgo et al., 2018). Mutations in the exon 17 D816V is seen in patients with leukemia, these patients will be resistant to imatinib treatment. A great number of GIST patients eventually stop responding to imatinib treatment. This can happen due to a secondry mutation taking place in the ATP/imatinib binding pocket that inhibits imatinib from binding into the kit protein kinase. Patients who develop resistance

could become candidates for another tyrosine kinase inhibitor. Sunitinib (SU11248) is another inhibitor of vascular epidermal growth factor recptor-2, pdgfra and kit. This inhibitor has been approved by FDA for treating GIST patients who have developed imatinib resistance.

RET

Newer types of therapeutic approaches which include tyrosine kinase inhibitors, monoclonal antibodies targeting oncogenes, gene therapies with dominant negative RET mutants, and nuclease-resistant aptamers which hinder RET, have been made. These new plan has shown evidence selective cancer therapies can be used to potentially target RET. Although, a therapeutic option which is clinally useful for treating RET-associated cancer patients is still not available (de Groot, Links, Plukker, Lips, & Hofstra, 2006). ZD6474, which is an anilino-quinazoline and has anti-angiogenic effects through inhibition of VEGFR shows some promising results (Dang, Reddy, Shokat, & Soucek, 2017).

RAS

There are several approaches that are now in clinical trials which aim to inhibit K-ras activity. These inhibitors are usually very toxic for human cells. Aminobiphosphonates are currently showing promising effects. They are now under clinical trials for the cure of bone metastasis and several other neoplasms including prostate adenocarcinomas and breast cancer (M. et al., 2006).

Mutations of the K-ras genes has a crucial affect in the targeted treatment of EGFR. They are also involved with the progression of resistance of EGFR inhibitors in non-small-cell lung cancer (Dang et al., 2017).

BRAF

BRAF gene is now used as a new target for the drugs which are used to treat melanomas. Small molecules and oligonucleotides are used as inhibitors to block kinase activity of MAP kinases in BRAF, inhibit BRAF/ras interaction and also block the BRAF protein expression. Sorafenib is one of these inhibitors which has entered phase III clinical trials (Dang et al., 2017).

AKT

The radioresistance and Akt drug response in many tumors is associated with Akt expression. It helps in the development of resistance to hormonal therapies in ovarian cancer. It also causes the development of resistance to cisplatin in caspase-dependent mitochondrial death pathways by modulating the direct effects of p53. Small molecule inhibitors have been developed which target Akt, such as pyridine and tricirine derivatives and inhibitors or small molecules. However, there has been complications due to the toxicity caused by Akt in insulin signaling pathways (Dang et al., 2017).

PIK3CA

PIK3CA acts as a target for many drug for its involvement in the development of carcinogenesis. The drugs which have been developed to target PIK3CA are highly toxic to human cells. Still, some have entered clinical trials. Mutations of PIK3CA are linked to the development of resistance to trastuzumab and cetuximab in breast cancers and colorectal cancer, respectively (Dang et al., 2017).

BCL-2

Antiapoptotic bcl-2 members are targets for developing anticancer drugs to provoke cell death. The mechanisms by which this class of drugs are; using oligonucleotides for downregulating the expression of bcl-2 or by using small organic molecules or peptides to bind to the bcl-2 pockets and prevent the proapoptotic proteins. The mechanism by which these drugs work is different than other cytotoxic drugs and radiation therapies. Thus, allowing this class of drugs to be combined with other treatments to create a synergetic effect without creating toxicity and resistance (Dang et al., 2017).

## 3.3 Drugs Targeted for Oncogenes

For thirty years, personalized therapies have been used for the cure of cancer. Recently, the concept of oncogenes in the development of cancer has provided greater insight in the complexity of tumor progression and to develop better targeted drugs for improving patient outcomes (McDermott & Settleman, 2009). For instance, imatinib has drastically helped in the diagnosis of gastrointestinal stromal tumors and chronic myeloid leukemia by targeting and blocking c-KIT tyrosine kinase domains and the abnormal fusion protein Bcr-Abl, respectively (Gambacorti-Passerini, 2008). In recent clinical methods, personalized cancer therapies can be used for several predictive biomarkers. The overexpression of HER-2, mutations of EGFR and K-ras, and methylation of methyl guanine methyl transferase (MGMT) are measured routinely to aid in the selection of the most suitable treatment plan for cancer patients. A potential biomarker which can be used as a target to treat hematologic and solid tumors is the anaplastic lymphoma kinase TK receptor (Grande, Bolos, & Arriola, 2011).

In around 50% of the human cancers, the MYC family oncogenes are deregulated. It is involved with poor diagnosis and low rates of patient survival. The myc gene has a crucial function in oncogenesis. It is responsible for metabolism, differentiation, apoptosis and

*Figure 3: Targeting Myc for treatment of cancer*

A number of strategies are developed to target myc. CDK9, CDK7 and BRD4 inhibition is used to suppress the expression of MYC at the transcriptional levels. mTOR/AKT/PI3K pathway inhibition can block the translation of MYC. On the other hand, PLK1, AURKA, USP7 inhibitors can cause the destabilization of myc at posttranslational level. Myc–Max dimeric complex is inhibited by 10058-F4 and Omomyc function. Rapamycin is targeted by CDK7 cyclin-dependent kinase 7, BRD4 bromodomain-containing 4, CDK9 cyclin-dependent kinase 9, PI3K/AKT/mTOR phosphatidylinositol 3-kinase/AKT/mammalian and PLK1 polo-like kinases 1 (H. Chen et al., 2018).

Small molecules

Compounds with low molecular weight ranging below 900 daltons are called small molecules. Specific proteins inside the cells can be targeted by small molecules (Joo et al., 2013; Nwibo et al., 2015). Inhibition of signaling pathways and kinases which are associated in the progression of cancer are caused by small molecules. Additionally, PARP inhibitors, CDKs and proteasomes are targeted by small molecules for the activation of apoptosis, DNA repair mechanisms and cell-cycle checkpoints (Lheureux et al., 2017) (Table 3).

Signaling pathways which regulate angiogenesis, migration and cell growth and proliferation are modulated by kinases (Gerber, 2008). Cell growth can be abrupted by abnormal regulation of these kinase proteins (Dobashi et al., 2012). Inhibitors of small molecule attach competitively to both the inactive and active ATP binding sites of the tyrosine kinases, thus impacting the tumor cells (Abramson, 2017; Dobashi et al., 2012) (Fig. 3). An example is a 2-phenylaminopyrimidine molecule called Gilvec. Gilvec causes competitive inhibition of

ATP binding to Abelson tyrosineprotein kinases and is the first tyrosine kinase inhibitor which was permitted for the cure of cancer in 2001 (Capdeville et al., 2002). Another drug which became popular for inhibiting disorders of the B-cell is Bruton‘s tyrosine kinase (BTK). BTK is involved in the migration, survival, proliferation, maturation and differentiation of the B-cells. BTK also targets the Toll-like receptor and B-cell receptor pathways (Mullard, 2017; Hendriks et al., 2014). Two BTK inhibitors were accepted by the FDA, acalabrutinib and ibrutinib, for their success in the treatment of B-cell malignancies (Mullard, 2017).

Activators of small molecules are also responsible for activating anti-cancer mechanisms for personalized therapies. SMBA3, SMBA2 and SMBA1 are small molecule Bax agonists which cause dephosphorylation of Bax at S184 regions and restores the pro-apoptotic feature in lung cancers (Xin et al., 2014). Activators of small molecules can also cause the induction of pyruvate kinase 2 and cause the induction of serine auxotrophy. This leads to cytostasis in human adenocarcinomic alveolar basal epithelial (A459) cancer cells (Kung et al., 2012). A small molecule which activates p53 is NSC146109. It does so by restoring the function of the p53 gene by inhibition of the expression of double minute X. This leads to apoptosis of the breast cancer cells (Wang and Yan, 2011). The procaspase-activating compound 1 has the potential to chelate labile inhibitory zinc ions from procaspase-3 to help in the stimulation of procaspase-3 to caspase-3. This results in the inhibition of tumor growth by apoptosis, commonly in lymphoma and osteosarcoma (Botham et al., 2016). McClinch and his associates have shown that DT-1154 and TRC-794 are activators of small molecules which attach and stimulate the protein phosphatase 2A (PP2A) which is a tumor suppressive actions in prostate cancer (McClinch et al., 2014). Small molecule inhibitors are currently only used in clinics. Thus, the capability of activators of small molecule in the cure of cancer treatment remains to be an emerging topic of research. Listed below is the list of small molecules along

with their target and cancer type which have been approved by FDA and are used in clinics (Table 11).

*Table 11: List of small molecule inhibitors approved by FDA that are used in clinic*

| Small molecules | Target | Cancer type |
|---|---|---|
| Tyrosine kinase inhibitors | | |
| Gefitinib | EGFR | Non-small cell lung cancer |
| Erlotinib | EGFR | Non-small cell lung cancer |
| Lapatinib | EGFR/ ERBB2 | ERBB2-positive breast cancer |
| Sorafenib | VEGFR kinase, RAF, PDGFR | Renal cancer |
| Crizotinib | ALK kinase | |
| Sunitinib | VEGF and PDGFR, SCF | Non-small cell lung cancer<br>Gastrointestinal stromal tumor |
| Pazopanib | VEGFR, PDGFR, FGFR, SCF | Advanced renal cell carcinoma |
| Imatinib | PDGFR<br>ABL kinase | Advanced soft tissue sarcoma |
| Acalabrutinib | BTK inhibitor | |
| Ibrutinib | BTK inhibitor | Advanced renal cell carcinoma<br>Chronic mylogenous leukemia<br>Gastrointestinal stromal tumors<br>Mantle cell lymphoma<br>Chronic lymphocytic leukemia |
| Proteasome inhibitors | | |
| Carfilzomib | Proteasome | Multiple Myeloma |
| Bortezomib | Proteasome | Multiple Myeloma |
| Ixazomib | Proteasome | Multiple Myeloma |
| Cyclin-dependent kinase | | |

| | | |
|---|---|---|
| (CDK) inhibitors<br>Ribociclib<br>Palbociclib | CDK4, CDK6 | Metastatic breast cancer |
| Poly ADP-ribose polymerase (PARP) inhibitors<br>Rucaparib<br>Olaparib<br>Niraparib | PARP | BRCA-positive ovarian cancer, gBRCA-mutated advanced ovarian cancer, Epithelial ovarian, fallopian tube, or primary peritoneal cancer |

Therapeutic monoclonal antibodies

As monoclonal antibodies (mAbs) are too big to pass through the cells, they are produced as targets which are situated outside the cells (Joo et al., 2013). Hybridoma technology is used to develop mAbs. The pioneers of this technique are Köhler and Milstein in 1975 (Köhler and Milstein, 1975). It was not used in humans until early 1980s (Houghton and Scheinberg, 1986). Extracellular proteins are specifically targeted by mAbs specifically for the inhibition of tumor growth my hindering the interactions between ligands and receptors. These antibodies have an indirect or direct action by attaching to the cancer cells (Weiner et al., 2010). The direct mechanism is related to the attaching of mAbs to membrane-bound proteins, cell receptors or antigens to directly impose their actions on the specific targets and to cause induced cell death (van de Donk et al., 2015). Indirect mechanism is related to the consecutive response of the body‘s defence mechanisms by recruiting effector cells or stimulating by attachement of the mAbs to the specific antigens of the cancer cells (Foltz et al., 2013). The latter approach is more commonly used in immunotherapy to target cancer cells. mAbs can give their action by a number of mechanisms through cytotoxic payload delivery, apoptosis induction, signal transduction

blockage, complement dependent cytotoxicity, antibody-dependent cellular phagocytosis and antibody dependent cellular cytotoxicity (Suzuki et al., 2015; van de Donk et al., 2015). For instance, trastuzumab is used to prevent HER2 protein directly by HER2 internalization triggering, dimerization or degradation. It can also indirectly recruit CD16-mediated antibody dependent cellular cytotoxicity in breast cancer cells. This inhibits division of cancer cell, receptor internalization promotion and cell cycle arrest induction (Luque-Cabal et al., 2016; Vu and Claret, 2012). mAbs can also use a arrangement of different mechanisms to induce antitumour effects.

Another highly selective, highly specific and potent therapy that is used in cancer treatment is antibody-drug conjugates (ADC). ADCs are composed of monoclonal antibodies which are target specific. They are covalently bonded to small molecule anticancer drugs. They act by targeting receptor-mediated endocytosis to release cytotoxins which causes the cancer cells to undergo apoptosis (Peters and Brown, 2015). The FDA has accepted a number of ADCs as cancer treatments. These include inotuzumab ozogamicin (BESPONSA, Wyeth Pharmaceuticals Inc., a subsidiary of Pfizer Inc) for the cure of adults with relapsed or refractory B-cell precursor acute lymphoblastic leukemia, adotrastuzumab emtansine (KadcylaVR) for metastatic breast cancer and brentuximab vedotin (AdcetrisVR) for Hodgkin lymphoma and anaplastic large cell-lymphoma. Other ADCs are currently undergoing clinical trials (Abdollahpour-Alitappeh et al., 2018; Beck et al., 2012, 2011). Combination of monoclonal antibodies with cytotoxic agents has been identified to enhance drug pharmacokinetic profiles by cause the decrease distribution volume and also elongating the eliminating and distribution phases (Dosio et al., 2011). Listed below are the monoclonal antibodies which are used to inhibit cancer along with their specific targets (Table 12).

*Table 12: Monoclonal antibodies which inhibit critical cancer targets*

| Monoclonal Antibody | Target/ Tumor Marker | Cancer Type |
|---|---|---|
| Brentuximab vedotin (SGN-35; Adcetris®) | CD30 | Hodgkin‘s lymphoma (HL) and systemic anaplastic large cell lymphoma (ALCL) |
| Adotrastuzumab emtansine (KadcylaVR) | HER2+ | HER2-positivemetastatic breast cancer (MBC) |
| Y-Ibritumomab tiuxetan (Zevalin®) | CD20 | non-Hodgkin‘s lymphoma (NHL) |
| Bevacizumab (Avastin®) | VEGF | Metastatic colorectal cancer (mCRC), nonsquamous, Nonsmall cell lung cancer (NSCLC), glioblastoma, and metastatic renal cell carcinoma. |
| (Lemtrada®) | | Chronic lymphocytic leukemia (CLL) |
| Nivolumab (Optivo) | CD52 | NSCLC, RCC, hepatocellular carcinoma (HCC) |

Therapeutic cancer vaccine

Therapeutic cancer vaccines are used to target immune-mediated antitumour responses. This can be classified into two key groups: patient-nonspecific and patient-specific. The patient‘s own tumor cells are used to generate patient-specific vaccines, while induction of the general immunologic response are used to generate patient-nonspecific vaccines, these vaccines possess antitumour effect in a small number of patients (Bilusic and Madan, 2012). Tumor-associated antigens are specifically targeted by therapeutic cancer vaccines through stimulation of T-cell and cause the induction of antitumour immune response. The frequency of tumor-associated antigens expression in cancer, its specificity to tumor cells as compared to normal cells and the intensity of tolerance to the given TAA are essential for therapeutic cancer vaccines effectiveness (Schlom et al., 2014). For example, EGFRvIII, a tumour-restricted neo-antigen EGFR variant III, which is an antigen involved with glioblastoma multiforme (GBM) (Sampson et al., 2010a). Mutation of EGFRvIII stimulated in GBM (Sampson et al., 2010a). In phase II clinical trials, 82% of GBM patients having EGFRvIII expression responded positively to PEPvIII. This led to the absence of expression of EGFRvIII on GBM and an enhanced rate of patient survival (Sampson et al., 2010b). The main proteins which are targeted by therapeutic cancer vaccines are the RAS and HER2 oncoproteins, tissue lineage, viral proteins, oncofetal antigens and stem cells (Schlom et al., 2014).

Gene therapy

DNA or RNA genetic materials are introduced into the cancer cells in order to inhibit their growth and proliferation by the use of gene therapies. Gene therapies are carried out by substituting the mutated tumour suppressor gene with normal functioning gene or by inhibiting the oncogene expression by introducing the antisense oligonucleotide or siRNA which stimulate immune responses inhibit angiogenesis processes (National Cancer Institute, 2013). The methodology which are used in gene therapies are oncolytic virotherapy, RNA

interference, immunotherapy, aptamers, microenvironment modulation, targeted genomic interventions, oligonucleotide therapeutics, gene transfer and gene editing (Sadelain, 2016).

The "Clustered Regularly Interspaced Palindromic Repeats" technique, also known as CRISPR or CRISPR-Cas9, is a novel genome editing tool for gene therapy. The ability of CRISPR-Cas9 to be used in genome editing was first recognized by Jinek and associates. It was done through the detailed study on the ability of the Cas9 endonuclease's site-specific cleavage to bring in a targeted double-stranded break in Streptococcus pyogene bacteria (Jinek et al., 2012). This technique was also experimented in human cells (Jinek et al., 2013) by the use of a single-guide RNA (sgRNA) libraries. This technique is used for elucidation for studying the function of genes which are a part of the development and progression process of diseases and their correction with the mutations which cause the disease, control of tumor suppressor genes and oncogenes, and also for identifying drug-target and the disease-resistance genes (Jiang and Doudna, 2016). Generally, the CRISPR spacer sequence is transcribed into a short RNA sequence. This RNA sequence functions as a guiding molecule. Consecutively, the Cas9 protein, which is a CRISPR-associated endonuclease binds to the mutated sequence of DNA and excises it. This results in gene silencing (Jinek et al., 2012). Recently, a study conducted by Chen and colleagues has accounted for prosperous uses of CRISPR-Cas9 to present a gene which codes for herpes simplex virus type 1 thymidine kinase (HSV1-tk) to change a mutated DNA of the cancer fusion gene with a suicide gene that induced cell death of the human prostate cancer and hepatocellular carcinoma cells (Chen et al., 2017). In an experimental study, CRISPR technology was used to inhibit the gene of the PD-1 protein and reactivate the T-cells to attack the cancer cells in multiple myelomas melanomas and sarcomas. This concluded that CRISPR can be used for both clinical applications and research purposes in tumour-specific lymphocytes (Su et al., 2016).

# Chapter 4

# Conclusion

The development of human neoplasia is a multistep process which involves changes taking place in genetic levels in somatic cells. These genetic alterations lead to oncogene activation and tumor suppressor genes inactivation. Oncogenes rise from mutations of the proto-oncogenes. Proto-oncogenes are a genes which are responsible in the cell growth regulations. The purposes of these genes include, programmed cell death regulation, transcription factors, signal tranducters, growth factors. Proto-oncogenes are converted to oncogenes by gene amplification, chromosomal rearrangements and mutations. Translocations and inversions are the two types of chromosomal rearrangements. By these two processes, proto-oncogenes are activated by gene fusion and transcription deregulation. Tumor suppressor genes account for maintaining normal cell growth. Inactivation of tumor suppressor genes by point mutations and alterations in their protein sequence leads to the loss of their functional activity.

A better insight about the genetic basis and development of cancer was understood after the discovery of the role of oncogenes. Oncogenes are also responsible for regulating normal cell differentiation, proliferation, growth and cell death. Identifying the abnormalities associated with oncogenes has provided useful tools to be used in the monitoring and diagnosis of cancer. Most impotantly, oncogenes are also possible targets for the development of new drug therapies. These new drugs can be used to selectively target cancer cells without causing any harm to human cells. A potential approach could be to use specific oncogene targets to induce cell death. An example of this approach was presented by the inhibition of bcr/abl tyrosine kinase in CML by imatinib (Druker et al., 2001). Imatibin has also shown promosing effects in the inhibition of c-kit in the treatment of

GIST (Heinrich, Blanke, Druker, & Corless, 2002) and also in chordomas, where it inhibits PDGFR (Casali et al., 2004). Another example is presented by C-225 and gefitinib, which inhibits the extracellular domain and intracellular tyrosine kinase, respectively, of the erb B gene.

However, oncogene-targeted therapies have three important issues. The first being, the concept of oncogene addiction. Oncogene addiction is when in most tumors, a single mutation confers for the unique biological properties and develops the oncogenic phenotype. So, the tumor cells become dependent on the specific type of mutation for their survival (Raffaella Sordella, Daphne W. Bell, Daniel A. Haber, & Jeffrey Settleman, 2004). Secondly, these genomic drivers only represent a small segment of patients with different solid tumors (Arnedos, Soria, Andre, & Tursz, 2014). And thirdly, the high number of genomic changes that are validated clinically to be predictive markers are the genes which code for the molecules which are involved in the pathways related to the sustenance of proliferation or inhibition of apoptosis (Hanahan & Weinberg, 2011). The only exceptions are the tumors which carry trunk mutations in the genes which are involved in the mechanisms of DNA repair. For example, BRCA1 mutations have been validated successfully as predictive markers for poly ADP ribose polymerase inhibitors in ovarian carcinomas.

Predicting the functional consequences of the chromosomal-scale structure variation is challenging. Interpretation is simplified in cases where mutations change the gene structures. But in most cases, interpreting the genomic changes are difficult. The distinction of passenger from functional mutations in noncoding regions of the human genome is an open frontier. ENCODE is a research consortium which was used to identify myriad functional elements of the DNA can be used to interpret the functional roles of various coding regions in the DNA. The methods which are used to make these predictions are not

yet developed, however approaches are being made to code for the sequences (Chapman et al. 2011). There have been promising results from the time the war with cancer has started. The decade after the completion of the human referencing genome has given some outstanding findings, particularly the discovery of the genes that are responsible for cancer development. Probably, the greatest challenge for the future may be putting these discoveries into action by turning them into successful treatment strategies. Still today, cancer targeted therapies are very limited (Wheeler & Wang, 2013).

Nevertheless, a range of new targeted drugs have recently entered clinical trials, (Table 3) with promising benefits for the treatment of a number of neoplastic diseases that were previously hard to treat. With the discovery and use of high-throughput technologies, identification of newer oncogenes has increased. Also, the increasing knowledge of the mechanisms of cancer have provided us with better insight for the development of improved cancer therapies for the upcoming future (Zugazagoitia et al., 2016).

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