Eukaryon

Volume 6 *Evolving Connections* Article 31

3-1-2010

Lung Cancer: The Breathtaking Battle of TKIs and EGFR Mutants

Robert Hodges
Lake Forest College

Elizabeth Ross
Lake Forest College

Natalie Simak
Lake Forest College

Keith Solvang
Lake Forest College

Eliot Vildaver
Lake Forest College

Lung Cancer: The Breathtaking Battle of TKIs and EGFR Mutants

Robert Hodges, Elizabeth Ross, Natalie Simak, Keith Solvang, Eliot Vildaver*
Department of Biology
Lake Forest College
Lake Forest, Illinois 60045

Summary

Lung cancer is the number one cause of cancer-related death involving excessive cell growth in epithelial tissues lining the lungs. Non-small cell lung cancer (NSCLC) is the most prevalent form of lung cancer. Genome-wide association studies provide insight into NSCLC. Single nucleotide polymorphism variations on chromosomes 15q24 and 15q25 lead to increased risk of NSCLC. Also, two tyrosine kinase receptors, epidermal growth factor receptor (EGFR) and human epidermal growth factor receptor 2 (HER2 / ERBB2), have mutated kinase domains associated with NSCLC. Proteins with Src homology 2 (SH2) and phosphotyrosine domains interact with EGFR and ERBB2, allowing proteins to bind to phosphotyrosine residues. Two of these proteins containing phosphotyrosine residues are vascular endothelial growth factor-A (VEGF-A) and platelet-derived growth factor receptor- ß (PDGFR-ß). These are present in the PI3K/Akt pathway involving angiogenic proteins p85 and p110. There are several drugs that inhibit these signaling pathways. Gefitinib inhibits the phosphorylation of mutant EGFR and ERBB2 receptors. Multiple mutations on kinase domains of EGFR and ERBB2 lead to higher Gefitinib sensitivity. Imatinib decreases phosphorylation of PDGFR-ß and VEGF-A, reducing tumor cell proliferation and cisplatin activates a signal-transduction pathway causing apoptosis of tumor cells involving p53 and p73 signaling pathways.

Introduction

Non-small cell lung cancer (NSCLC) is a carcinoma, or a malignant cancer that arises from epithelial tissue (University of Maryland, 2008). NSCLC is a disease involving uncontrolled cell growth in tissues lining the lungs, which could result in metastasis or the spread of disease into nearby tissue beyond the lungs (Chen et al., 2009). Because of its high rate of metastasis, NSCLC accounts for nearly 80% of all lung cancers and is the leading cause of cancer-related deaths worldwide (Jemal et al., 2008). Despite the lung cancer medical advances made, mortality rates are still high, with an overall five year survival of only 15% (Jemal et al., 2008).

There are three major types of NSCLC: squamous cell carcinoma, which affects the skin, mouth, esophagus, and lungs, large cell carcinoma named after the large appearance of its cells under the microscope, and adenocarcinoma, which begins in cells lining the alveoli (University of Maryland, 2008). These different classes of NSCLC are named based on the types of cells they contain and how they look under a microscope. There are also minor forms of NSCLC, including pleomorphic, carcinoid tumor, and salivary gland carcinoma (University of Maryland, 2008).

*This author wrote the paper for Biology 221: Cell and Molecular Biology taught by Dr. Shubhik DebBurman.

Many risk factors are associated with NSCLC. Tobacco is the number one risk, accounting for nearly 90% of all incidences (Johnson et al., 2008). Each one year delay in starting to smoke a fixed daily cigarette decreases an individual's lung cancer risk by 10% (Franks & Teich, 1986). However, there are other risk factors including exposure to radiation therapy, asbestos, radon, arsenic, and air pollution.

Many times patients with NSCLC do not experience any symptoms. They are unaware that they have the disease until they undergo routine chest x-rays. If symptoms of lung cancer are present, they are caused by either a primary tumor or by metastatic cancer cells. A primary tumor is one which begins in the original organ and has not metastasized. Primary tumors usually damage surrounding tissues, blood vessels, or nerves (Alberg, Ford, & Samet, 2007). Metastatic lung cancer may cause the same problems, but in different areas of the body. The primary symptoms of non-small cell lung cancer include persistent coughing, wheezing, shortness of breath, chest pain, difficulty swallowing, hoarseness, loss of appetite, and recurrent respiratory infection (Johnson, 2009). These symptoms are very common, and it is important to get tested if any of them are persistent.

There are several factors that affect the prognosis and treatment of non-small cell lung cancer. These include the size of the tumor, how far the cancer has metastasized, and the overall health of the patient (Schiller et al., 2002). There is currently no cure for NSCLC, and it is recommended for patients with all stages of NSCLC to take part in extensive clinical trials. Treatments for early-stage NSCLC include various types of surgery and radiation. Chemotherapy can be used to treat both early-stage and advanced NSCLC (Schiller et al., 2002). Treatment with Avastin and platinum-based chemotherapy involving cisplatin has been used since 2006 as a first-line treatment option for patients with advanced, non-squamous cancer (Hall et al., 2008).

The molecular mechanisms involved in NSCLC are still relatively unknown and are being investigated extensively. Over the last decade, extensive research has been performed on tyrosine kinases, which are enzymes that can transfer a phosphate group from ATP to tyrosine residues, or amino acids, of proteins (Engelman et al., 2007). Biological agents known as tyrosine kinase inhibitors, such as Gefitinib and Imatinib, are being studied as potential treatments of NSCLC (Engelman et al., 2007). These particular drugs block small, mutated protein kinase molecules involved in the growth of blood vessels that ultimately help promote tumor growth in the cell. These drugs have the potential to inhibit specific growth factors and their receptors, including EGFR, ERBB2, PDGFR-β, and VEGF-A. Researchers believe that by turning off these mutated protein molecules as well as the signaling pathways associated with them, inhibition of tumor cell proliferation will result.

Implications of Chromosomal Loci in the Development of Lung Cancer

Genome-Wide Association Studies and SNPs

The recent completion of sequencing of the Human Genome has already begun to pay dividends in disease research. Through the use of genome-wide association studies, breakthroughs have been seen in the pathogeneses of many genetically complex diseases including diabetes,

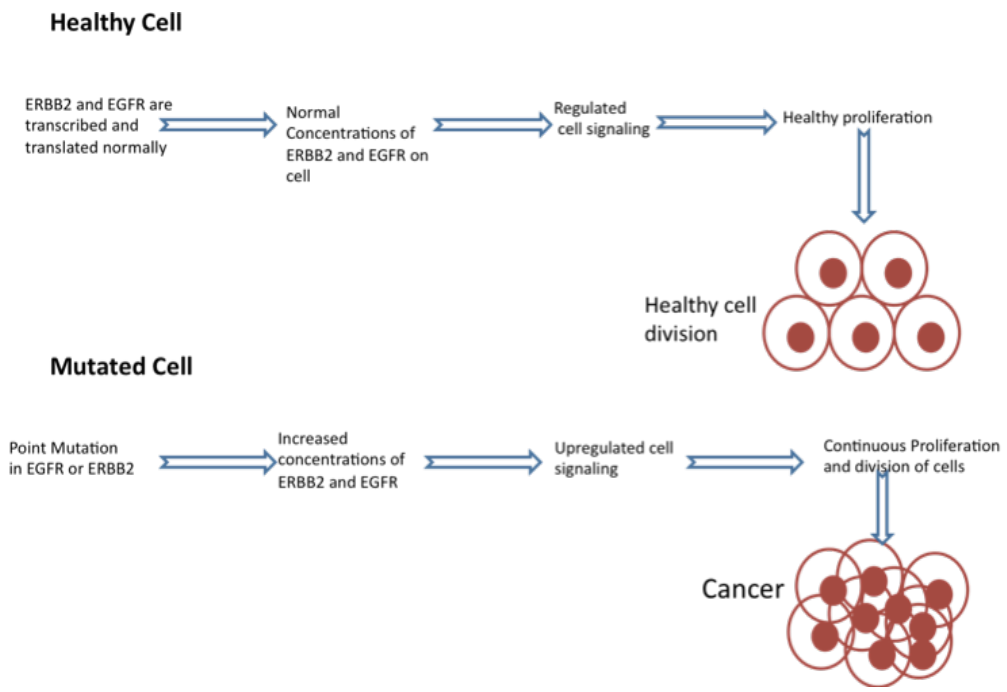

Figure 1. Properties of a healthy and mutated cell. This figure depicts the events that occur from the transcription and translation of EGFR and ERBB2 until the activation and signaling of the cell to proliferate. A) Represents the absence of a mutation in EGFR and ERBB2, thus a regulated signal and healthy proliferation and division pursue. B) Depicts a point mutation in EGFR and ERBB2 and thus the upregulation of the proliferation signal and constant division leading to cancer.

inflammatory bowel disease, heart disease, and cancer (Hung et al., 2008). Genome-wide association studies examine genetic variations amongst a particular genome, characterizing observed genotypic characteristics to observed phenotypes. Genome-wide association studies allow researchers to study large samples, which make these studies a key component to solving the mysteries of modern diseases.

Recently, genome-wide association studies have provided great insight into the development of lung cancer. These studies have pinpointed specific loci on chromosomes that are strongly associated with risk of the disease. Single-nucleotide polymorphisms (SNPs) are contained within these loci, which account for differences in a genome (Hung et al., 2008). SNPs are single nucleotide variations in the DNA sequence that differ from that of the majority of the population. It is in these SNPs that variations occur which allow researchers to be able to associate specific SNPs and chromosomal loci to the development of lung cancer (Hung et al., 2008).

Chromosomal Culprits

Three known chromosomal culprits have already been identified as being highly associated with the development of lung cancer: 15q24, 15q25, and 5p15.33 (Chanock & Hunter, 2008). The 15q24 and 15q25 regions house many genes including nicotinic acetylcholine receptor subunits (Chanock & Hunter, 2008). Nicotinic acetylcholine receptor subunits stimulate acetylcholine-activated ion-channel-linked receptors in neurons when nicotine binds to them (Alberts et al., 2004). The genes found on the 5p15.33 region include the human telomerase reverse transcriptase (TERT) (McKay et al., 2008). Human telomerase reverse transcriptase is a protein subunit that catalyzes the enzyme telomerase, which is responsible for the addition of telomeres to the end of replicating DNA strands (Alberts et al., 2008).

Genome-wide association studies were utilized to identify various SNPs associated with the risk of lung cancer on these loci. The SNP rs8034191 on region 15q25 was most prevalent amongst lung cancer cases, far exceeding the genome-wide significance level (Hung et al., 2008). On the 5p15.33 region, the SNP rs402710 was most frequently associated with increased risk of the disease. (McKay et al., 2008). Interestingly, an SNP, rs1051730 was found on region 15q24 to not only be highly associated with risk of developing lung cancer, but also with high smoking quantity within lung cancer patients (Thorgeirsson et al., 2008). This link between smoking behavior and lung cancer provides a somewhat extraordinary gene-environment interaction.

Gefitinib Interacts With EGFR/ERBB2 Pathway

Receptor tyrosine kinases are proteins within cells that regulate various signaling pathways involved in critical cellular activities such as growth, motility, survival, and apoptosis (Pedersen et al., 2005). One tyrosine kinase receptor important in cancer is known as epidermal growth factor receptor (EGFR). Signaling of this membrane-bound receptor involves an intricate pathway of ligand binding, receptor homodimerization, and heterodimerization with ERBB2 and other family members, followed by internalization and recycling of the ligand-bound receptor or ubiquitin-mediated receptor degradation (Fig. 1A) (Kwak et al., 2005). EGFR is frequently found overexpressed or mutated in many human malignancies including those of the brain, breast and lung (Pedersen et al., 2005). This overexpression stimulated the development of specific pharmacological tyrosine kinase inhibitors such as gefitinib.

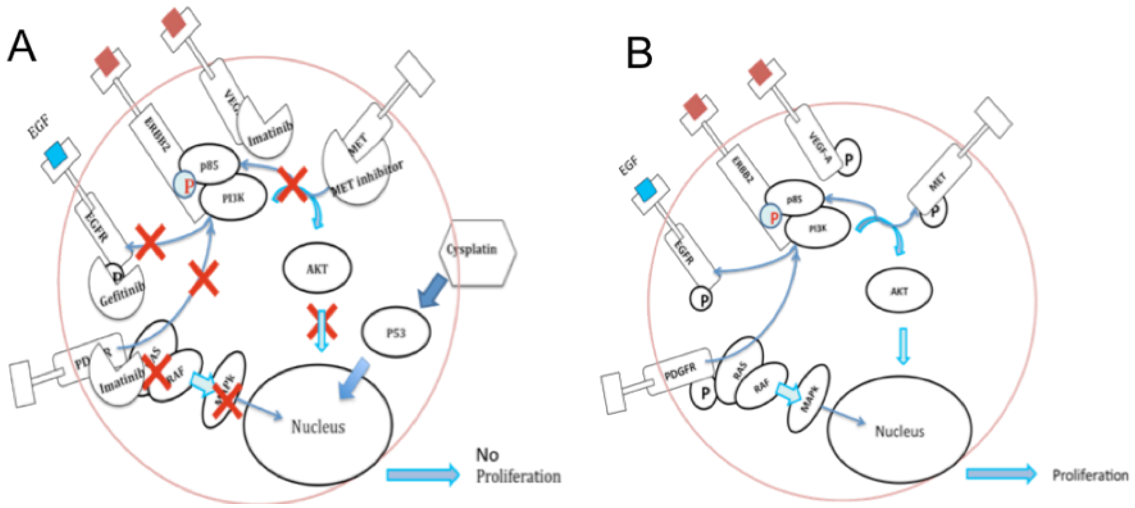

Figure 2. New Biological Model: Pathways Leading to NSCLC and Treatment Options. A) Depicts the increased concentrations of ERBB2, EGFR, MET, PDGFR, and VEGF due to genetic mutations along with decreased affinity for the substrates, causes a proliferation signal to continuously be sent to the nucleus as seen. B) Application of gefitinib, imatinib, and cisplatin results in the inhibition of tumor cell proliferation. An MET inhibitor can also be used to inhibit cell proliferation.

Gefitinib is an orally administered drug that disrupts EGFR kinase activity by binding to the ATP-binding pocket of the EGFR protein (Pedersen et al., 2005). This binding prevents the heterodimerization between EGFR and a family member of ErbB. Heterodimerization is essential in order to start the cascade of signals that lead to cell growth, and in the cases with mutated EGFR, excessive cell proliferation.

Independent studies have been conducted to determine the effects of gefitinib on patients with NSCLC. It was found that gefitinib could suppress the tyrosine phosphorylations of cells expressing EGFR mutants (EGFRvIII) more effectively than the wild-type EGFR (Chen et al., 2006). Interestingly, the concentration of gefitinib affects its inhibition capabilities. Research has shown that long term exposure to high concentrations of gefitinib (1-2 μm) is needed to stop phosphorylation and signaling of PLC-y and AKT by EGFRvIII cells when compared to wt-EGFR (Pedersen et al., 2005). However, when low concentrations of gefitinib (0.01-0.1μm) were introduced, it appeared that the drug actually stimulated receptor autophosphorylation, proliferation and anchorage-independent growth of the EGFRvIII cells (Pedersen et al., 2005). While this finding was consistent in all mutated EGFR cells, higher doses of gefitinib equated to a greater inhibition rate of EFGRvIII cells; it also showed a surprising result that researches could not quite explain. Higher doses of gefitinib worked, but its ability to inhibit growth varied depending on the type of EFGR mutation.

EGFR Mutations Do Not Have to be Located in the Kinase Domain to be Sensitive to Gefitinib

Mutations in the kinase domain of EGFR are associated with clinical responsiveness to gefitinib in patients with NSCLC (Chen et al., 2006). The most common oncogenic mutations found in patients with non-small cell lung cancer are small, in-frame deletions on exon 19 and the point mutation L858R (Yun et al., 2007). After many experiments, researchers found that gefitinib could suppress the tyrosine phosphorylation of most EGFR mutants better than the wild-type receptor (Chen et al., 2006). However, this research also found that gefitinib has variable growth-suppressive effects on different EGFR mutant-expressing cells (Chen et al., 2006). It has been suggested that the increased binding affinity between mutant receptors and the inhibitor was the major cause of hypersensitivity to gefitinib (Chen et al., 2006). However, this claim is no longer considered valid. In 2006, researchers used mutations that were located at distinct positions in the EGFR kinase domain and found that all of the mutations had a higher sensitivity to gefitinib (Chen et al., 2006). Even more interesting, researchers found that the EGFRvIII mutant lacking a mutation in the kinase domain was more sensitive to gefitinib than the wild type EGFR (Chen et al., 2006). These two pieces of evidence propose that a mutation in EGFR kinase domain is not essential for obtaining hypersensitivity to gefitinib and further suggests that hypersensitivity to gefitinib in mutated EGFR could possibly be derived from other properties that are introduced by the mutations (Chen et al., 2006).

Classes of Proteins That Are Associated in Lung Cancer

Phosphorylation of many membrane- bound proteins can lead to the activation of these proteins' kinase function which sends a signal through consequent proteins by phosphorylation. Three amino acids have the ability to be phosphorylated: serine, threonine, and tyrosine. Specifically, these proteins are phosporylated at a tyrosine residue (amino acid). The signal can then be sent through receptor protein/kinases such as PI3K/p85, Akt, or STAT 5. (Rikova et al., 207). These proteins contribute to the rest of the pathway and must be phosphorylated to continue the signal. Specific domains, such as the Src homology 2 and phophotyrosine binding domain, bind to the phosphorylated tyrosine residue (Jones et al., 2006). Once these proteins are activated, then the different signals, such as apoptosis, proliferation, and cell division, are sent to the nucleus to be activated (Jones et al., 2006).

EGFR and ERBB2 contributions

The EGFR (ERBB1) signaling pathway is one of the most known and well studied epidermal growth factor pathways that contribute to the development of lung cancer (Jones et al., 2006). Some of the cell processes that are signaled

include apoptosis, cell cycle progression, and some even in adhesion signaling (Shilo & Yarden, 2007). Like EGFR, mutations in ERBB2 have been well studied. Typically, mutations in these genes cause an increase in concentration of ERBB2 and EGFR (Figure 1B). Consequently, the proliferation signal of the cell is continuously activated because when the concentrations of these proteins increase, their affinity thresholds for ligands decrease (Jones et al., 2006). EGFR protein mutations represent 10% of all NSCLCs while ERBB3 protein mutations account for about 5% (Rikova et al., 2007). This may seem minor, but it is important when other proteins such as PI3KA and B-RAF only account for 5% together (Rikova et al., 2007). Thus, the 15% of NSCLC caused by EGFR and ERBB2 protein mutations are significant.

Promiscuous Proteins

Although EGFR and ERBB2, together, contribute to about 15% of NSCLC, there are many more membrane bound proteins that contribute to mutations. However, before 2005, many of these proteins were not studied or even known about contributing to cancer (Rikova et al., 2007). Like EGFR, these proteins are kinases that can be activated by binding with the correct ligand and can then send the specific signal down the pathway using the Src domain and PTB domain as well (Jones et al., 2006). Over 20 different amplified proteins were found in one or more of the 11 cell lines and 12 tumor samples (Rikova et al., 2007). Specifically, of the 20 amplified proteins, MET, PDGFRa, ALK, and VEGF were most abundant (Figure 2A) (Rikova et al., 2007). These four proteins were found in more than five cell lines and 10 tumor samples each. Over 22 different cell lines and 96 tumor samples from NSCLC patients, each containing more than 50% cancerous cells, were analyzed (Rikova et al., 2007). This is significant because these proteins are all normally found in healthy cells; however, amplification of these proteins and continuous signaling is a characteristic of cancer. Interestingly, the various proteins were not amplified in the same samples.

The expression of these proteins may be cause by the abundance of EGFR and ERBB3 in cancerous cells. As their concentrations increase, they begin to bind more frequently and send their specific signal continuously through the cell (Jones et al., 2007). The ability for a cell to increase expression of different proteins and continuously activate many different pathways leads to decreased sensitivity toward treatments. Drugs such as gefitinib, an EGFR inhibitor, as well as MET inhibitors, will no longer be able to be used as independent treatments. Instead, a cocktail of gefitinib and MET inhibitors might be necessary. When EGFR is inhibited by gefitinib, MET may become amplified due to a secondary mutation and, therefore, inhibition of MET is required. Both drugs would then be necessary as treatments to stop the signaling pathways of MET and EGFR (Engelman et al., 2007). Depending on the type of NSCLC, more drugs, such as lamitinib-a PDGFR receptor inhibitor, may be used as well (Figure 2B) (Rikova et al., 2007). This drug would stop the phosphorylation of the membrane-bound kinases, thus halting the continuous signal to proliferate through the cell.

Signal Transduction Mechanisms

The PI3K/Akt Pathway

The PI3K/Akt signaling pathway is linked to the growth, survival, migration, and invasion of malignant cancer cells. Evidence points to the importance of deregulated PI3K/Akt signaling in lung cancer (Chen et al., 2009). The activation of growth factor receptor protein kinases results in autophosphorylation on tyrosine residues and transphosphorylation of adaptor proteins on tyrosine (Ruddon, 2007). Phosphoinositide 3-kinase (PI3K) is brought to the membrane and activated by binding to phosphotyrosine residues of growth factor receptors or adaptors (Figure 2A) (Ruddon, 2007). The lipid product of PI3K, phosphatidylinositol-3,4,5-triphosphate (PIP3), obtains a subset of signaling proteins to the membrane where they become activated (Ruddon, 2007).

One of these proteins is protein kinase B, or serine-threonine kinase Akt. Serine-threonine kinase Akt is the key downstream effector of PI3K, and it phosphorylates and regulates the activity of a number of targets in response to the activation of PI3K (Chen et al., 2009). Serine-threonine kinase Akt and a number of other proteins initiate the complex set of events that control protein synthesis, cell cycle entry, cell survival, and actin polymerization. A very important regulatory step in the Akt pathway is the dephosphorylation of PIP3 to PIP2 by the protein phosphatase and tensin homolog, or PTEN, which is encoded by the PTEN gene (Ruddon, 2007). Overall, inhibition of PI3K/Akt activation and disruption of the associated proteins are implicated as promising approaches for lung cancer treatment (Chen et al., 2009). Feline Models of HCM: MYBPC3 Mutations

In 2005, a research team lead by Dr. Kathryn Meurs discovered the first spontaneously occurring mutation causing heritable HCM in a non-human species. The mutation was found in a family of Maine Coon cats that had been previously diagnosed with the disease. HCM in this colony of cats was shown to closely resemble the human form by Kittleson et al. in 1999. They found that, consistent with HCM in humans, these cats presented varying phenotypes, from mild to severe (Kittleson et al., 1999). These results lead to the proposal that affected cats are a valuable, naturally occurring animal model for studying the cellular, molecular, and anatomical aspects of HCM and may be an important resource for studying possible therapeutic treatments of the condition (Kittleson et al., 1999).

In the study by Meurs et al. (2005), affected Maine Coon cats were determined to have a causative mutation in exon 3 of the feline ortholog of the MYBPC3 gene. The mutation, a single base pair change of G to C, modified the amino acid sequence of the cMyBP-C protein, which was predicted to result in an alteration in protein structure. This abnormal conformation may explain the sarcomeric disorganization and increased left ventricular wall thickness (range = 6-9 mm, normal = 3-5mm) observed in these cats. Furthermore, affected cats were found to have decreased levels of the cMyBP-C protein in their myocardium in comparison to control cats ($p < 0.001$). This may indicate that the mutated proteins are degraded due to their instability and their inability to integrate into the sarcomere, supporting the previously mentioned findings by Sarikas et al. (2005).

More recently, a second mutation in the feline MYBPC3 gene was identified in a similar population of Ragdoll cats with HCM (Meurs et al., 2007). The mutation caused similar phenotypes, but was in a different domain than the Maine Coon mutation. This suggests that the two mutations were independent and not the result of a common founder. The mutations resulted in a change from arginine to tryptophan in the protein's amino acid sequence due to a single base pair change from C to T in codon 820, yielding an abnormal protein product. These discoveries in feline models of HCM provided more evidence for the importance of the MYBPC3 gene in cardiac structure and function and illustrate that it is a key component of hypertrophic cardiomyopathy in cats as well as humans.

Tyrosine Kinase Pathways and Protein Phosphatases
Tyrosine kinase proteins are a large group of signal transduction proteins that include cell surface receptors for growth factors such as EGFR, ERBB2, PDGFR, and VEGFR (Moyer et al., 1997). There are a large number of cytoplasmic tyrosine kinases that activate downstream effectors and control a number of developmental, cell proliferation, and cell differentiation pathways (Ruddon, 2007). The p85-α and p110-α subunits activate enzymes producing secondary messengers. Mutations usually lead to altered tyrosine kinase pathways and are observed in the PI3K signaling pathway. These changes ultimately decide whether activation or induction of nuclear transcription factors, to turn genes on or off, occurs. Phosphatase proteins play a major role in the activity of various receptors and particular cell cycle-regulating genes. Protein tyrosine phosphatases (PTPases) are a very unique group of enzymes present in the cell membrane, and typically associate with tyrosine kinase receptors (Ruddon, 2007). Evidence has shown that PTPases are involved in NSCLC. One in particular, PTEN, is considered to be a tumor suppressor because previous studies have shown that loss of PTEN activity increases PIP3 phosphorylation by PI3K (Chen et al., 2009).

Angiogenesis

Vascular Endothelial Growth Factor
Angiogenesis is a normal process in which new blood vessels are formed for the development of many cells. This process is highly regulated, being turned on for specific periods of time and then shut off. However, in the case of tumor angiogenesis, the response is unregulated and results in many diseases. Angiogenesis is an important step in tumor cell proliferation because it creates an independent blood supply for the cancer cell (Helotera & Alitalo, 2007). It was not known until recently that endothelial cells produce VEGF, which is essential for angiogenesis to occur in cells. VEGF was originally thought to be only a tumor vascular permeability factor, but it is a major mediator of angiogenesis as well (Chen et al., 2009). Important to angiogenesis is autocrine VEGF, which follows a signaling pathway whereby a cell secretes a hormone or chemical messenger that binds to receptors on the same cell, causing intracellular changes (Alberts et al., 2004).

VEGF is necessary for pathological growth of vessels in many diseases including NSCLC and investigations have been targeted to suppress VEGF signaling (Helotera & Alitalo, 2007). VEGF acts through both tyrosine kinase receptors VEGFR-1 and VEGFR-2, with VEGFR-2 being the major mediator of angiogenesis (Ferrara & Kerbel, 2005). Angiogenesis is required for metastasis of tumor cells, which must have increased expression of various metastasis-promoting genes. VEGF-A expression, a potent angiogenesis promoter, is observed in almost all aggressive tumors. VEGF-A also stimulates observed cell migration and invasion of cancers cells throughout various organs of the body by activating the PI3K/Akt pathway (Chen et al., 2009).

FLJ10540 Protein
FLJ10540 is a protein that may function as an oncogene in the development of tumors, and it has been shown in a recent study that it is required for necessary VEGF-A-dependent signaling in the PI3K pathway. High levels of FLJ10540 help induce AKT phosphorylation and form a complex with PI3K (Chen et al., 2009). A recent experiment suggests that FLJ10540 is overexpressed in lung cancer tissues, and this is in positive correlation with the overexpression of VEGF-A, which leads to hyperactivation of the PI3K/Akt pathway (Chen et al., 2009). The overexpression of FLJ10540 thus contributes to cell changes and tumor cell proliferation through the activation of this pathway. In addition, FLJ10540 serves a critical role in enhancing cell migration and invasion, resulting in enhanced metastatic potential of cancer cells. There is an elevated upregulation of FLJ10540 in tumor lung tissue, indicating that there are both migratory and invasive qualities of lung cancer cells (Chen et al., 2009).

Platelet-Derived Growth Factor
Angiogenesis relies on more than solely VEGF-A to function. Platelet-derived growth factor (PDGF) has angiogenic effects in vitro and in vivo. Specifically, PDGF-β acts on pericytes covering blood vessels, which then secretes Ang1 to stabilize the formation of blood vessels (Helotera & Alitalo, 2007). Angiopoietin-1 (Ang-1) is a protein that has an important role in blood vessel maturation and stability. This contributes to vascular development and angiogenesis as a whole.

Current Drug Treatments for NSCLC: Tyrosine Kinase Inhibitors

Imatinib is a specific PDGFR-ß inhibitor that inhibits the function of phosphorylated PDGFR- ß (p-PDGFR-ß), a tyrosine kinase receptor. It also decreases interstitial fluid pressure in cells, downregulates VEGF, and improves tumor oxygenation, which was shown in recent studies to be beneficial (Figure 2B). A major challenge related to drug delivery in tumor cells is related to the heterogeneous blood supply. In angiogenesis, excessive growth of blood vessels in tumors leads to extensive tumor interstitial fluid pressure (TIFP), which is marked by a reduced delivery of anticancer drugs (Vlahovic et al., 2006). High levels of IFP indicate solid, or malignant, tumors. Phosphorylated PDGFR-ß is a target for lower TIFP in tumors that overexpress PDGFR-ß. Imatinib treatment is shown to reduce IFP within tumors (Vlahovic et al., 2006). There is also improved tumor oxygenation with the use of Imatinib. Tumor hypoxia limits the curability of tumors because it involves depriving tumor cells of oxygen necessary for cytotoxic effects of radiation and chemotherapy. One cause of this is the inability of antitumor agents to penetrate beyond more than 50-100 μm from capillaries (Vlahovic et al., 2006). Another reason is that hypoxic tumor cells divide very slowly, and chemotherapy and radiation target rapidly-dividing cells (Vlahovic et al., 2006). Finally, the downregulation of VEGF is present in Imatinib treatment, thus inhibiting the process of angiogenesis.

Anti-Cancer Drug: Cisplatin
Although there is no known cure for NSCLC, clinical trials have led to a variety of treatment options including platinum (Pt) based drugs. Effectiveness of the Pt drug is related to its accumulation within a cell. Resistance to current anticancer treatments has been linked to poor cellular absorption of the drug. The amount of Pt based drug entering cells is increased compared to these other drugs. Pt based drugs cross the cell membrane via either passive diffusion or active uptake by transport proteins (Hall et al., 2008). Once inside the nucleus, the Pt-containing drug functions by creating crosslinks within DNA (Poklar et al., 1996). A cis-Pt-GG intrastrand crosslink is formed when two adjacent N-7 guanine bases coordinate with the platinum (Ohndof et al., 1999). As a result of the crosslink, the Pt-DNA adduct unwinds and bends the DNA. Therefore, the two DNA strands cannot properly separate and the processes of replication and transcription become impaired (Hall et al., 2008).

One such Pt anticancer drug is cis-diamminedichloridoplatinum(II), also known as cisplatin (CDDP). After CDDP has modified the DNA of a cell, the Pt-DNA adduct activates a signal-transduction pathway which leads to apoptosis. Two distinct pathways act in CDDP induced cell death: a p53 and a p73 pathway. P53 is a tumor suppressor; similarly, p73 is a homologue of p53. The p53 pathway can be induced by CDDP as well as a variety of DNA-damaging agents such as ultraviolet radiation (Figure 2B) (Gong et al., 1999). The p73 pathway is induced by CDDP via the nuclear enzyme non-receptor tyrosine kinase c-Abl (White & Prives, 1999). In order for c-Abl to induce p73, it must first become activated by a DNA damaging agent, such as CDDP (Yuan et al., 1999).

Expression of c-Abl led to an increase in the amount of p73 but not p53, indicating that the presence of c-Abl is required for p73 to function optimally. Further evidence for this was the co-expression of c-Abl and p73 but not p53 (Gong et al., 1999). C-Abl is activated when it is phosphorylated by a protein kinase known as ataxiatelangiectasia-mutated (White & Prives, 1999). The absence of c-Abl disrupts the G1-S checkpoint in response to DNA damage while the activation of c-Abl allows p73 to accumulate within the cell and induce apoptosis (Gong et al., 1999).

In order for p73 to initiate cell cycle arrest, the Pt damaged cell must interact with a high mobility group domain (HMG). HMG-domain proteins, including p73, are attracted to the Pt-DNA adduct and bind to the DNA at a phenylalanine residue on the crosslinked site of the Pt-DNA adduct. Mutant cells, in which alanine is substituted for phenylalanine, have reduced binding of the HMG- domain to the DNA and, therefore, display reduced sensitivity to CDDP (Ohndorf et al., 1999). Studying these interactions is necessary to design new anticancer drugs with increased affinity for Pt-modified DNA.

Conclusion

Non-small cell lung cancer is a carcinoma arising from epithelial tissues and is characterized by uncontrolled cell growth in the tissues lining the lungs. Specific loci that contain single-nucleotide polymorphisms (SNPs) cause differences in the human genome that lead to the development of lung cancer. SNPs located at chromosomes 15q24, 15q25, and 5p15.33 are associated with the development of lung cancer. The SNP on region 15q25 is the most prevalent in lung cancer. Tyrosine kinase receptors are proteins that regulate signaling pathways involved in growth, motility, survival, and apoptosis of cells. Epidermal growth factor receptor is overexpressed or mutated in lung cancer along with ERBB2 in a heterodimerization essential to start the cascade of cell proliferation signals. Not only is EGFR and ERBB2 overexpressed in NSCLC, but in over 20 different membrane bound proteins including MET, PDGFR, and VEGF. VEGF is important because it induces the PI3K pathway to stimulate angiogenesis, which allows the tumor to grow. Treatments can block the ability for EGFR to become self-phosphorylated, as the case with gefitinib, or function as PDGFR and VEGF inhibitors (Imatinib). Another type of drug, cisplatin, can induce p53 and p73 to cause the cell to apoptose. Thus, there are many different kinds of mutations that can occur in NSCLC. Some can occur from smoking, such as EGFR and ALK mutations. However, some mutations can occur spontaneously such as the ROS, MET, and PDGFR mutations (Rikova et al. 2007). This means that current treatments may have to be used in a cocktail to account for all the different possible protein mutations. To further study the use of the cocktail of drugs, trials must be moved from in vitro to in vivo within animal models. Consequently, mutations can occur even after one tyrosine kinase is inhibited. Thus, new research must be done to discover further secondary mutations. The next step is to further study associations between amplified proteins in resistant NSCLC cell lines and tumor samples. Further research needs to be conducted into the process of metastasis with regards to NSCLC. Finally, we need to take a deeper look at other drugs that may lead to a destruction of the tumor cells themselves. For example, as mentioned before, Cisplatin is a platinum-based drug that kills the cancer cell. Although very toxic, these drugs need to be researched further because they by-pass further secondary mutations and are able to destroy the cancerous cells.

Acknowledgements

We would like to thank Dr. Sleiter for her guidance and support in writing this review article. Also, we would like to thank Dr. DebBurman for his time and patience. We are grateful to Michael Fiske for reviewing our paper and for his invaluable comments.

References

Abid MR, Guo S, Minami T, Spokes KC, Ueki K, et al. (2004) Vascular endothelial growth factor activates PI3K/Akt/forkhead signaling in endothelial cells. Arterioscler Thromb Vasc Biol 24: 294-300.

Agami, R., Blandino, G., Oren, M., and Shaul, Y. (1999). Interaction of c-Abl and p73alpha and their collaboration to induce apoptosis. Nature 399, 809-813.

Alberg AJ, Ford JG, Samet JM; American College of Chest Physicins. Epidemiology of lung cancer: ACCP evidence-based clinical practice guidelines (2nd edition). Chest. 2007; 132:29S-55S.

Alberts, B., Bray, D., Hopkin, K., Johnson, A., Lewis, J., Raff, M., Roberts, K., and Walter, P. 2004. Essential Cell Biology. Garland Science. New York, pp740.

Chanock, Stephen J., Hunter, David J. 2008. When the smoke clears. Nature. 452: 537-538.

Charron, P., Dubourg, O., Desnos, M., Bennaceur, M., Carrier, L., Camproux, A., et al. (1998). Clinical features and prognostic implications of familial hypertrophic cardiomyopathy related to the cardiac myosin-binding protein c gene. *Circulation, 97*, 2230-2236.

Chen, C.H., Lai, J.M., Chou, T.Y., Chen, C.Y., Su, L.J., Lee, Y.C., Cheng, T.S., Hong, Y.R., Chou, C.K., Whang-Peng, J., Wu, Y.C., and Huang, C.Y. (2009). VEGFA upregulates FLJ10540 and modulates migration and invasion of lung cancer via PI3K/AKT pathway. PLoS ONE 4, e5052.

Chen, Y.R., Fu, Y.N., Lin, C.H., Yang, S.T., Hu, S.F., Chen, Y.T., Tsai, S.F., and Huang, S.F. (2006). Distinctive activation patterns in constitutively active and gefitinib-sensitive EGFR mutants. Oncogene 25, 1205-1215.

Engelman, J.A., Janne, P.A., Mermel, C., Pearlberg, J., Mukohara, T., Fleet, C., Cichowski, K., Johnson, B.E., and Cantley, L.C. (2005). ErbB-3 mediates phosphoinositide 3-kinase activity in gefitinib-sensitive non-small cell lung cancer cell lines. Proc. Natl. Acad. Sci. U. S. A. 102, 3788-3793.

Ferrara, N. and Kerbel, R.S. (2005). Angiogenesis as a therapeutic target. Nature 438, 967.

Franks, L.M. and Teich, N. (1986). Introduction to the Cellular and Molecular Biology of Cancer (New York:Oxford University Press).

Fox, P., Liu, S.K., & Maron, B. (1995). Fox, P., Liu, S.K., & Maron, B. (1995). Echocardiographic assessment of spontaneously occurring feline hypertrophic cardiomyopathy: An animal model of human disease. *Circulation, 92,* 2645-2651.

Gao, B., Lee, S.M., and Fang, D. (2006). The tyrosine kinase c-Abl protects c-Jun from ubiquitination-mediated degradation in T cells. J. Biol. Chem. 281, 29711-29718.

Gautel, M., Zuffardi, O., Freiburg, A., & Labeit, S. (1995). Phosphorylation switches specific for the cardiac isoform of myosin binding protein-C: a modulator of cardiac contraction? *The EMBO Journal, 14* (9), 1952-1960.

Granzier, H., & Campbell, K. (2006). New insights in the role of cardiac myosin binding protein c as a regulator of cardiac contractility. *Circulation Research, 99*, 795-797.

Hall, M.D., Okabe, M., Shen, D.W., Liang, X.J., and Gottesman, M.M. (2008). The role of cellular accumulation in determining sensitivity to platinum-based chemotherapy. Annu. Rev. Pharmacol. Toxicol. 48, 495-535.

Harris, S., Bartley, C., Hacker, T., McDonald, K., Douglas, P., Greaser, M., Powers, P., Moss,R. (2002). Hypertrophic cardiomyopathy in cardiac myosin binding protein-C knockout mice. *Circulation Research, 90*, 594-601.

Helotera, H. and Alitalo, K. (2007). The VEGF Family, the Inside Story. Cell 130:591-592

Hung, Rayjean J. et al. 2008. A susceptibility locus for lung cancer maps to nicotinic acetylcholine receptor subunit genes on 15q25. Nature. 452: 633-637.

Huang, J.C., Zamble, D.B., Reardon, J.T., Lippard, S.J., and Sancar, A. (1994). HMG-domain proteins specifically inhibit the repair of the major DNA adduct of the anticancer drug cisplatin by human excision nuclease. Proc. Natl. Acad. Sci. U. S. A. 91, 10394-10398.

Jemal A, Siegel R, Ward E, Hao Y, Xu J, et al. (2008) Cancer statistics, 2008. CA Cancer J Clin 58: 71-96.

Johnson DH, Blot WJ, Carbone DP, et al. Cancer of the lung_Non-small cell lung cancer and small cell lung cancer. In: Abeloff MD, Armitage JO, Niederhuber JE, Kastan MB, McKena WG. Clinical Oncology. 4th ed. Philadelphia, Pa: Churchill Livingstone Elsevier: 2008: chap 76.

Jones, R.B., Gordus, A., Krall, J.A., and MacBeath, G. (2006). A quantitative protein interaction network for the ErbB receptors using protein microarrays. Nature 439, 168-174.

Kittleson, M., Meurs, K., Munro, M., Kittleson, J., Liu, S.K., Pion, P, & Towbin, J. (1999). Hypertrophic cardiomyopathy in Maine coon cats: An animal model of human disease. *Circulation, 99*, 3172-2180.

Kulikovskaya, I., McClellan, G., Flavigny, J., Carrier, L., & Winegard, S. (2003). Effect of MyBP-C binding to actin on contractility in heart muscle. *Journal of General Physiology, 122*, 761-774.

Kulikovskaya, I., McClellan, G., Levine, R., & Winegrad, S. (2007). Multiple forms of cardiac myosin—binding protein c exist and can regulate thick filament stability. *Journal of General Physiology, 129* (5), 419-428.

Kwak, E.L., Sordella, R., Bell, D.W., Godin-Heymann, N., Okimoto, R.A., Brannigan, B.W., Harris, P.L., Driscoll, D.R., Fidias, P., Lynch, T.J. et al. (2005). Irreversible inhibitors of the EGF receptor may circumvent acquired resistance to gefitinib. Proc. Natl. Acad. Sci. U. S. A. 102, 7665-7670.

Lever, H., Karam, R., Currie, P., & Healy, B. (1989). Hypertrophic cardiomyopathy in the elderly. Distinctions from the young based on cardiac shape. *Circulation, 19*, 580-589.

Marian, A.J., & Roberts, R. (2001). The molecular genetic basis for hypertrophic cardiomyopathy. *Journal of Molecular and Cellular Cardiology, 33*, 655-670.

Maron, B.J. (2002). Hypertrophic cardiomyopathy: A systematic review. *JAMA, 28* (10), 1308-1320.

Maron, B.J., Tajik, A., Ruttenberg, H., Graham, T., Atwood, G., Victorica, B., Lie, J., & Roberts, W. (1982). Hypertrophic cardiomyopathy in infants: clinical features and natural history. *Circulation, 65*, 7-17.

McKay, James D. et al. 2008. Lung cancer susceptibility locus at 5p15.33. Nature Genetics. 40(12): 1404-1406.

Meurs, K., Sanchez, X., David, R., Bwoles, N., Towbin, J., Reiser, P., Kittleson, J. et al. (2005). A cardiac myosin binding protein c mutation in the Maine Coon cat with familial hypertrophic cardiomyopathy. *Human Molecular Genetics, 14* (23), 3587-3593.

Meurs, K., Norgard, M., Ederer, M., Hendrix, K., & Kittleson, M. (2007). A substitution mutation in the myosin binding protein C gene in ragdoll hypertrophic cardiomyopathy. *Genomics, 90*, 261-274. doi: 10.1016/j.ygeno.2007.04.007

Moyer JD, Barbacci EG, Iwata KK, Arnold L, Boman B, et al. (2006) Induction of apoptosis and cell cycle arrest by CP-358,774, and inhibitor of epidermal growth factor receptor tyrosine kinase. Cancer Res 57: 4838-4848.

National Center for Biotechnology Information (2007). Homo sapiens complex locus MYBPC3, encoding myosin binding protein C, cardiac. *AceView: A Comprehensive cDNA-supported gene and transcripts annotation.* Retrieved February 14, 2008 from http://www.ncbi.nlm.nih.gov/IEB/Research/Acembly/av.cgi?exdb=AceView&db=36a&term=MYBPC3&submit=Go

Niimura, H., Patton, K., McKenna, W., Soults, J., Maron, B., Seidman, J., & Seidman, C. (2002). Sarcomere protein gene mutations in hypertrophic cardiomyopathy of the elderly, *Circulation, 105*, 446 - 451.

Oakley, C., Hambly, B., Curmi, P., & Brown, L. (2004). Myosin binding protein C: Structural abnormalities in familial hypertrophic cardiomyopathy. *Cell Research, 14* (2), 95-110.

Ohndorf, U., Rould, M., He, Q., Pabo, C., Lippard (1999). Basis for recognition of cisplatin-modified DNA by high-mobility-group proteins. Nature. 399: 708-712.

Pedersen, M.W., Pedersen, N., Ottesen, L.H., and Poulsen, H.S. (2005). Differential response to gefitinib of cells expressing normal EGFR and the mutant EGFRvIII. Br. J. Cancer 93, 915-923.

Pietras K, Ostman A, Sjoquist M, Buchdunger E, Reed RK, Heldin CH, Rubin K (2001) Inhibition of platelet-derived growth factor receptors reduces interstitial hypertension and increases transcapillary transport in tumors. Cancer Res 61: 2929-2934.

Poklar, N., Pilch, D.S., Lippard, S.J., Redding, E.A., Dunham, S.U., and Breslauer, K.J. (1996). Influence of cisplatin intrastrand crosslinking on the conformation, thermal stability, and energetics of a 20-mer DNA duplex. Proc.Natl. Acad. Sci. U. S. A. 93, 7606-7611.

Richard, P., Charron, P., Carrier, L., Ledeuil, C., Cheav, T., Picheereau, C., Benaiche, A., et al. (2003). Hypertrophic cardiomyopathy: Distribution of disease genes, spectrum of mutations, and implications for a molecular diagnosis strategy. *Circulation, 107*, 2227-2232. doi: 10.1161/01.CIR.0000066323. 15244.54.

Richard, P., Villard, E., Charron, P., & Isnard, R. (2006). The genetic bases of cardiomyopathies. *Journal of the American College of Cardiology*, *48* (9), A79-A89.

Rikova, K., Guo, A., Zeng, Q., Possemato, A., Yu, J., Haack, H., Nardone, J., Lee, K., Reeves, C., Li, Y. et al. (2007). Global Survey of Phosphotyrosine Signaling Identifies Oncogenic Kinases in Lung Cancer. Cell 131, 1190-1203.

Ruddon, R.W. (2007). Cancer Biology: Fourth Edition (New York: Oxford University Press).

Sadayappan, S., Osinska, H, Klevitsky, R., Lorenz, J., Sargent, M., Molkentin, J., Seidman, C., Seidman, J., & Robbins, J. (2006). Cardiac myosin binding protein c phosphorylation is cardioprotective. *PNAS, 103* (45), 16918-16923.

Sarikas, A., Carrier, L., Shenke, C., Doll, D., Flavigny, J., Lindenberg, K., Eschenhagen, T., & Zolk, O. (2005). Impairment of the ubiquitin-proteasome system by truncated cardiac myosin binding protein C mutants. *Cardiovascular Research, 66*, 33-44.

Schiller JH, Harrington D, Belani CP, Langer C, Sandler A, Krook J, Zhu J, Johnson DH (2002) Comparison of four chemotherapy regimens for advanced non-small-cell lung cancer. N Engl J Med 346: 92-98.

Seidman, J., & Seidman, C. (2001). The genetic basis for cardiomyopathy: From mutation identification to mechanistic paradigms. *Cell, 104*, 557-567.

Shilo, B., Yarden, Y. (2007). Snap Shot: EGFR Signaling Pathway. Cell.131, 1018e1-1018e2.

Stros, M., Ozaki, T., Bacikova, A., Kageyama, H., and Nakagawara, A. (2002). HMGB1 and HMGB2 cell-specifically down-regulate the p53- and p73-dependent sequence-specific transactivation from the human Bax gene promoter. J. Biol.Chem. 277, 7157-7164.

Thorgeirson, Thorgeir E. et al. 2008. A variant associated with nicotine dependence, lung cancer and peripheral arterial disease. Nature. 452: 638-642.

University of Maryland Medical Center. (Baltimore). University of Maryland School of Medicine. http://www.umm.edu/patiented/articles /non-small_cell_lung_cancer_000072.htm

Van Driest, S., Ommen, S., Tajik, J., Gersh, B., & Ackerman, M. (2005). Sarcomeric genotyping in hypertrophic cardiomyopathy. *Mayo Clinic Proceedings, 80* (4), 463-469.

Vlahovic G, Rabbani ZN, Herndon II JE, Dewhirst MW, Vujaskovic Z (2006) Treatment with Imatinib in NSCLC is associated with decrease of phosphorylated PDGFR-beta and VEGF expression, decrease in interstitial fluid pressure and improvement of oxygenation. Br J Cancer 95: 1013– 1019.

Weisberg, A., & Winegrad, S. (1996). Alteration of myosin cross bridges by phosphorylation of myosin-binding protein C in cardiac muscle. *PNAS, 93*, 8999-9003.

Yang, Q., Sanbe, A., Osinska, H., Hewett, T., Klevitsky, R., & Robbins, J. (1998). A mouse model of myosin binding protein c human familial hypertrophic cardiomyopathy. *Journal of Clinical Investigation, 102* (7), 1292-1300.

Yarden, Y., and Sliwkowski, M.X. (2001). Untangling the ErbB signalling network. Nat. Rev. Mol. Cell Biol. 2, 127-137.

Yuan, Z.M., Shioya, H., Ishiko, T., Sun, X., Gu, J., Huang, Y.Y., Lu, H., Kharbanda, S., Weichselbaum, R., and Kufe, D. (1999). p73 is regulated by tyrosine kinase c-Abl in the apoptotic response to DNA damage. Nature 399, 814-817.

Yun, C.H., Mengwasser, K.E., Toms, A.V., Woo, M.S., Greulich, H., Wong, K.K., Meyerson, M., and Eck, M.J. (2008). The T790M mutation in EGFR kinase causes drug resistance by increasing the affinity for ATP. Proc. Natl. Acad. Sci. U. S. A. 105, 2070-2075