Published in final edited form as:

Handb Exp Pharmacol. 2013 ; (216): 93–113. doi:10.1007/978-3-7091-1511-4_5.

Lung Cancer and Lung Injury: The Dual Role of Ceramide

Tzipora Goldkorn^{*}, Samuel Chung, and Simone Filosto

Center for Comparative Respiratory Biology and Medicine, Genome and Biomedical Sciences Facility, University of California School of Medicine, Davis, CA 95616, USA

Abstract

Sphingolipids play key roles in cancer, yet our current understanding of sphingolipid function in lung cancer is limited to a few key players. The best characterized of these are sphingosine-1-phosphate and ceramide which are described for their opposing roles in cell fate. However, because sphingolipids as a whole are readily interconverted by a complex enzymatic machinery, no single sphingolipid appears to have exactly one role. Instead, the roles of specific sphingolipids appear to be context specific as demonstrated by findings that ceramide-1-phosphate has both proliferative and apoptotic effects depending on its concentration. Therefore, we present herein several years of research on ceramide, a sphingolipid linked to apoptotic signaling, that is emerging in cancer research for its potential roles in proliferation and cell-to-cell communication via exosomes.

Ceramide is a well-studied sphingolipid in both normal and pathological conditions ranging from skin development to lung cancer. Interestingly, several groups have previously reported its increased levels in emphysema patients who are smokers, a patient subpopulation greatly susceptible to lung cancer. However, the molecular mechanisms through which cigarette smoke (CS) and ceramide accumulation lead to lung cancer, non-small cell lung cancer (NSCLC) specifically, are unknown.

Interestingly, recent studies clearly establish that two signaling pathways are activated during CS exposure in the lung airway. One centers on the activation of neutral sphingomyelinase2 (nSMase2), an enzyme that hydrolyzes sphingomyelin to ceramide. The other pathway focuses on the oncogenic EGF receptor (EGFR), which becomes aberrantly activated but not degraded, leading to prolonged proliferative signaling. Recent studies show that these two signaling pathways may actually converge and integrate. Specifically, Goldkorn et al. demonstrated that during CS exposure, EGFR is favorably co-localized in ceramide-enriched regions of the plasma membrane, proposing that nSMase2/ceramide plays a role in the aberrant EGFR activation, leading to augmented tumorigenic signaling. Moreover, new findings indicate that CS exposure may induce resistance to the tyrosine kinase inhibitors (TKIs), used for treatment of NSCLC, merely through posttranslational molecular alterations. Furthermore, structural anomalies of the CS-activated EGFR appear to be supported by the excess ceramide produced by the CS-activated nSMase2 in the plasma membrane of lung epithelial cells.

We present in this chapter the progression of the sphingolipid field in lung cancer using ceramide as an example. However, many crucial questions remain to be answered regarding the role of sphingolipids in lung cancer because of the glut of promising observations.

Keywords

Sphingolipids; Lung cancer and injury; Ceramide; EGFR; Neutral sphingomyelinase 2; Cigarette smoke; Oxidative stress

1 Introduction

It is well established that sphingolipids play crucial roles in a number of biological processes, including cell survival, proliferation, and apoptosis of many cell types. Consistently, it has been demonstrated that several different sphingolipids and sphingolipid-metabolizing enzymes are critical regulators of cancer cell fate. However, as of this moment, very little is known about the role of sphingolipids in lung cancer development; therefore, much more research and better models are required.

Indeed, despite the potential role of sphingolipids in several aspects of lung cancer biology such as cell growth and death, metastasis, and interaction/communication between cancer cells and their microenvironment, only a few fragmented investigations have been carried out to date. We present herein these studies together with our vision of the ceramide-generating machinery as a very promising, challenging, and still mostly undefined field of research that could lead to a paradigm shift and a better understanding of lung cancer. Particular emphasis is given to the potential function of ceramide in the context of smoking-related lung tumorigenesis, as tobacco smoking is the most prominent cause of lung cancer.

2 The Ceramide-Generating Machinery as the Center of Sphingolipids

Biology

Among the sphingolipids, ceramide has been one of the most studied compounds because it represents the core of the complex machinery, often referred to as “the ceramide-generating machinery,” which allows the conversion of one type of sphingolipid to another. Indeed, ceramide can be generated via at least three different pathways, namely, de novo, salvage, and sphingomyelin hydrolysis pathway (Mullen et al. 2011; Wu et al. 2010). At the same time, ceramide represents the main hub that provides many other sphingolipids, such as ceramide-1-phosphate, sphingosine, sphingosine-1-phosphate (S1P), and sphingomyelin, each of which can be involved in many and different biological processes (Hannun and Obeid 2011; Maceyka et al. 2012; Merrill 2011; Morales et al. 2007; Ponnusamy et al. 2010).

To date, the best-characterized sphingolipids in cancer cell fate are ceramide and S1P. These two lipids have been “canonically” described as harboring opposite roles in driving cell fate: ceramide often described as a pro-apoptotic or “tumor suppressor lipid” and S1P as pro-survival/-proliferation or “oncogenic lipid” (Oskouian and Saba 2010). However, this certainly oversimplifies the complex enzymatic machinery controlling sphingolipids

biology, where activation of specific enzymes can rapidly alter the balance between ceramide and S1P as well as trigger their conversion to several other bioactive ones. Moreover, recent evidences suggested that just amongst ceramides, different lengths of the fatty acid constituents of ceramide are associated with opposite effects on cell fate. For instance, it has been shown in various cancer models that C(16)-ceramide increases tumor growth, while C(18)-ceramide suppresses it (Ponnusamy et al. 2010), suggesting that different ceramide synthases may lead to opposite outcomes of cell fate.

Thus, it is important to point out that the effect of sphingolipids, particularly of ceramides, on cell fate (and thus on cancer growth/death) is the combined effect of several interconvertible sphingolipids which are regulated in distinct subcellular compartments, executing distinct cellular functions (Hannun and Obeid 2011).

3 Sphingolipids in Lung Cancer: A Field that Needs Research and Discovery

Our understanding of sphingolipids' role in lung cancer is still very limited and fragmented, perhaps suffering from the lack of suitable in vivo models to assess the long-term effects of certain sphingolipids or sphingolipid-regulating enzymes on tumor growth. Most studies on sphingolipids and lung cancer were performed in vitro, investigating short-term effects of sphingolipids on cell proliferation and apoptosis of lung cancer cell. Mitra et al. (2007) showed in A549 lung adenocarcinoma cells that ceramide-1-phosphate (C1P) can have either proliferative and pro-survival effects during serum starvation or pro-apoptotic effects depending on the concentration: low concentrations ($<5 \mu\text{M}$ C1P) leading to proliferation, whereas high concentration ($>5 \mu\text{M}$ C1P) causing apoptosis. In this study, Mitra et al. claimed that the cytotoxic effects of high C1P levels were due to ceramide accumulation, generated by dephosphorylation of C1P. On the other hand, the proliferative effects of C1P low concentration were attributed to reduced cell ceramide levels, possibly caused by inhibition of a sphingomyelinase (Gomez-Munoz et al. 2004; Mitra et al. 2007). Importantly, this study also showed that ceramide kinase expression directly correlated with enhanced cell growth. This strongly supports the notion that sphingolipids can be rapidly converted amongst themselves and that their overall effect on cell fate was a function of the prevailing sphingolipid-controlling enzymes that become activated (or inhibited) in a certain context.

Consistent with this idea, de Molina et al. (2012) reported that the conversion of ceramide to sphingosine by acid ceramidase is a mechanism regulating non-small cell lung cancer (NSCLC) resistance to targeted therapy (choline kinase- α inhibitors) despite an initial increase in ceramide levels. Importantly, this study highlights how sphingolipids can be critically involved in modulating lung cancer therapy resistance. Indeed, Schnitzer et al. (2009) recently showed that enhanced sphingosine kinase 2 expression, activity, and subsequent upregulation of sphingosine-1-phosphate (S1P) are part of the stress response to hypoxia in A549 cells, which was not only essential for cell viability but also protected cells from chemotherapy (etoposide)-induced apoptosis.

In the past, Inokuchi et al. (1990) proposed a potential role for several glycosphingolipids (glucosylceramide, lactosylceramide, ceramide trihexoside, globoside, and ganglioside GM3) in lung cancer, finding that glucosylceramide synthase contributes to metastatic spreading of Lewis lung carcinoma. Moreover, a recent study by Yamada et al. provides important evidence supporting both the role of sphingolipids in lung cancer growth and metastasis and the potential for effective therapeutic intervention by targeting sphingolipids. Their study showed high expression of the glycosphingolipid ganglioside GM2 (monosialic ganglioside 2) in small-cell lung cancer (SCLC) human specimens and, using an in vivo tumor xenograft mouse model, demonstrated that an anti-GM2 antibody-based therapy could block SCLC metastases and increase cancer cell apoptosis, thereby prolonging the mouse' survival (Yamada et al. 2011). However, as of yet, there are no molecular mechanisms explaining this observation.

Adding a level of complexity, many pathways can be activated downstream of bioactive sphingolipids which appear to be context specific. For instance, Mukhopadhyay et al. (2009) showed that membrane C(18)-ceramide can mediate its pro-apoptotic effects in lung cancer cells via its binding partner protein phosphatase 2A (PP2A), whose function is upstream of c-Myc degradation, thereby presenting antiproliferation effects. These findings are in line with earlier studies from the same group (Ogretmen and colleagues) showing that exogenous administration of C(6)-ceramide to A549 cells could inhibit both c-Myc and telomerase activity which are important for proliferation (Ogretmen et al. 2001). However, there could be many reasons why this PP2A-dependent apoptotic pathway is not undertaken or is not prevalent. For instance, Mukhopadhyay et al. (2009) also reported that okadaic acid treatment could completely counteract c-Myc degradation following exposure of A549 cells to exogenous ("pro-apoptotic") ceramide. Furthermore, this PP2A-dependent pathway relies on both low levels of its endogenous inhibitor I2PP2A and a functional PP2A. Since it has been shown that PP2A is inhibited by oxidative stress (Foley et al. 2007) and given that oxidative stress is a common denominator of many lung diseases and lung cancer, it is easy to envision that this PP2A-dependent apoptotic trigger would fail in lung cancer (Esme et al. 2008), particularly in the context of smoking-related lung cancer. Currently, much more research is needed to understand the role of sphingolipids in lung cancer. Moreover, future research should be oriented toward identifying bioactive sphingolipids in vivo: namely, models of lung cancer that better mimic the conditions where human lung cancer initiates and progresses.

To this extent, our group has been committed to investigate the cell signaling events initiated in lung epithelial cells exposed to cigarette smoke-induced oxidative stress, a model relevant to both smoking-induced lung injury and lung cancer (Filosto et al. 2011a; Goldkorn et al. 2005). Therefore, we will provide our perspective on sphingolipids research, with particular focus on ceramide signaling, in lung cancer.

4 Cigarette Smoke-Induced Lung Cancer and Lung Injury

Despite the well-documented dangers of smoking, millions of smokers continue to puff away every day, each puff containing roughly 5,000 toxic compounds that include 10^{15} free radicals such as hydrogen peroxide (H_2O_2) and hydroxyl and organic radicals in the gaseous

phase (Church and Pryor 1985). Toxic oxidants in cigarette smoke have a detrimental effect on epithelial cells lining the airway and contribute to several lung pathological conditions including emphysema and bronchitis [jointly known as chronic obstructive pulmonary disease (COPD)], which are injury-related lung diseases, and lung cancer. Indeed, tobacco smoking is the major risk for developing lung cancer, causing approximately 80–90 % of all cases (Alberg and Samet 2003).

Interestingly, several prospective clinical studies over the years show that lung cancer incidence becomes significantly increased in COPD patient populations despite that lung injury and lung cancer are apoptosis dominant and proliferation dominant, respectively. For instance, de Torres et al. (2007) reported that in their 5-year prospective cohort of former and current smokers, individuals with emphysema were more likely to develop lung cancer than those without emphysema. Moreover, individuals with both emphysema and airway obstruction were at an even higher risk than those with neither conditions alone (de Torres et al. 2007). Similarly, studies by Caballero et al. (2008) convincingly reported that even the existence of some low-grade emphysema without noticeable airflow obstruction is associated with significantly elevated risk of lung cancer.

Thus far, clinical data demands novel mechanistic insight into how apoptosis-dominant lung injury leads to proliferation-dominant lung cancer. Indeed, both smoking-induced lung injury and lung cancer, though originating from opposing phenotypes at the cellular level, may actually share common molecular mechanisms that underlie both pathologies and explain their clinical association. To respond to this major problem, we discuss herein the potential role for ceramide generation as a signaling event in both lung injury and lung cancer (Fig. 1).

5 Cigarette Smoke-Generated Oxidative Stress and Ceramide Generation in Airway Epithelial Cells

Epithelial cells lining both the lung main airways and the terminal alveoli represent the lung's first line of defense and thus are extensively exposed to reactive oxidants such as those present in the gas phase of CS. Over the last 15 years in our laboratory, we have been investigating the cell signaling events initiated in human airway epithelial (HAE) cells during CS exposure, leading to either apoptosis (hallmark of injury) or proliferation (hallmark of cancer).

Studies by Goldkorn et al. in the direction of oxidative stress-induced lung injury addressed whether HAE cells undergo apoptosis when exposed to micromolar concentrations of H_2O_2 and, subsequently, whether this process is mediated by ceramide generation (Chan and Goldkorn 2000; Goldkorn et al. 1998). Both a dose-dependent (50–250 μM H_2O_2) and a time-dependent relationship were observed between H_2O_2 exposure and increased cellular ceramide levels and subsequent apoptosis. This initial model for H_2O_2 -induced cellular oxidative stress and ceramide generation consisted of direct exposures of airway epithelial cells to H_2O_2 , glutathione (GSH), or both by media supplementation (Khan et al. 2006; Lavrentiadou et al. 2001; Ravid et al. 2003). Even though these early studies undertook a reductionist, simplified approach, both scenarios were relevant to the lung epithelium.

H₂O₂ is a ubiquitous molecule that is freely miscible and able to cross cell membranes readily and is present in several air pollutants, including the vapor phase of tobacco smoke (Khan et al. 2008). It is detected in exhaled air of humans (Williams and Chance 1983), and the amounts of exhaled H₂O₂ appear greater in subjects with pulmonary disease (Sznajder et al. 1989) and in cigarette smokers (Nowak et al. 1996). In addition, it was shown that either administration of exogenous H₂O₂ or enhancement of endogenously generated H₂O₂ was effective in depleting cellular GSH and initiating ceramide generation and apoptosis events (Ravid et al. 2003).

Interestingly, later studies showed that CS exposure regulates both growth and death of airway epithelial cells via its H₂O₂ component (Khan et al. 2008; Lavrentiadou et al. 2001): CS exposure could generate between 100 and 800 μ M H₂O₂, as measured in the media of cultured HAE cells, a factor that was dependent on the length of exposure and the amount of combusted tobacco. Furthermore, when the CS-generated H₂O₂ was quenched by antioxidants such as GSH or NAC (*N*-acetylcysteine), no ceramide upregulation was observed (Khan et al. 2008). Since then, it has been shown that exposure to oxidative stress leads to ceramide level increase prior to caspase-3-dependent apoptosis in airway epithelial cells, mimicking the dominant apoptotic process of emphysema pathogenesis (Ravid et al. 2003). Following this discovery, Goldkorn et al. sought to identify the enzymatic regulator(s) controlling this phenomenon.

6 Identifying Neutral Sphingomyelinase 2, a Novel Sphingomyelinase Upregulated in Cigarette Smoke-Induced Lung Injury

Sphingomyelin (SM) hydrolysis by sphingomyelinases (SMases) is considered a major pathway for cell stress-induced ceramide generation and subsequent apoptosis (Clarke et al. 2008; Filosto et al. 2010, 2011a; Goldkorn et al. 1998; Goldkorn and Filosto 2010; Nikolova-Karakashian et al. 2008; Ravid et al. 2003; Rutkute et al. 2007b; Wu et al. 2010). According to current models, SM may be metabolized to ceramide by several SMases, but some SMases may have special pathophysiological significance (Uhlig and Gulbins 2008).

Several years of purifying and cloning proteins from the lungs of nonhuman primates and HAE cells led to the independent identification of a novel sphingomyelinase, neutral sphingomyelinase 2 (nSMase2, SMPD3 gene), as the sole sphingomyelinase responsible for ceramide generation in response to oxidative stress (Levy et al. 2006). Importantly, it was demonstrated that nSMase2 is a redox-sensitive enzyme that is activated in HAE cells in response to ox-stress induced by H₂O₂ or by CS exposure (Levy et al. 2006, 2009). Moreover, silencing of nSMase2 expression using siRNA completely abrogates ceramide production and, more importantly, the downstream apoptotic events in response to oxidative stress. It was also demonstrated that the H₂O₂ component of the CS gas phase was responsible for the nSMase2 activation and ceramide generation (Levy et al. 2009).

Convincingly, both Goldkorn et al. and others have reported that the major SMase enzyme that becomes activated by ox-stress appears to be nSMase2 (Levy et al. 2006, 2009; Rutkute et al. 2007a). Last of all, but most notably, it has been shown that nSMase2 becomes overexpressed in human emphysema patients and rodents chronically exposed to cigarette

smoke and that mice heterozygous for nSMase2 (+/-) have dramatically reduced ceramide generation in response to CS (Filosto et al. 2011a).

Filosto et al. have since demonstrated a molecular mechanism that regulates nSMase2 function in response to ox-stress by finding that nSMase2 is controlled via a complex phosphorylation-based machinery. It was proven that nSMase2 phosphorylation on specific serines triggers both its activation and protein stabilization upon exposure of HAE cells to ox-stress (Filosto et al. 2012a). Furthermore, we identified some of the protein regulators of nSMase2 phosphorylation: Calcineurin (CaN) phosphatase binds directly to nSMase2 and dephosphorylates it, but not under ox-stress, when CaN is inhibited/degraded, allowing nSMase2 to be phosphorylated downstream of p38 mitogen-activated protein kinase (MAPK) and/or protein kinase C (PKCs) (Filosto et al. 2010) (Fig. 2).

7 Can nSMase2 Activation and Ceramide Generation Support Mitogenic Responses?

The above studies identified nSMase2 as the ceramide-generating enzyme during oxidative stress whose activation is a requisite to trigger apoptosis of HAE cells. However, several considerations should be taken before concluding that apoptosis is the sole downstream effect of ceramide generation. First, while increased ceramide levels could be expected for any cell exposed to ox-stress, only 20–50 % (depending on the exposure conditions) of the exposed cells would actually undergo apoptosis (Khan et al. 2008; Levy et al. 2009). Second, oxidative stress has also been proposed as a mitogenic signal promoting lung cancer growth (Khan et al. 2008; Levy et al. 2009). Third, most studies investigating ceramide generation under stress conditions, including ours, were conducted during acute, short-term exposure to cell stressors, and therefore, the long-term effects of ceramide generation on cancer cell growth have not been evaluated.

Presently, though it is plausible that during cellular stress ceramide acts as a direct stimulus for apoptosis, it is equally possible that ceramide generation has mitogenic effects favoring tumor development. Consistent with the latter, it has been recently shown that nSMase2 activity and subsequent ceramide generation under TNF- α -induced cell stress can be mechanistically linked to both apoptotic initiation as well as proliferative signaling (Devillard et al. 2010). While alternative pathways could lead to apoptosis when nSMase2 function was blocked, nSMase2 was required for the mitogenic response to TNF- α . An additional recent publication claims that loss of ceramide transfer protein (CERT) augments EGF receptor signaling in breast cancer via its regulation of cellular pool of sphingomyelin (SM). Reduction of CERT expression led to reduced SM levels, thereby enhancing tumorigenesis (Heering et al. 2012). Furthermore, nSMase2 and ceramide generation could be instrumental in the spreading of oncogenic proteins and miRNAs via exosomal generation, as described below.

8 nSMase2-Dependent Ceramide Generation and Exosome Secretion: A Vehicle for Propagation of Oncogenes and miRNA

The conventional view of communication between cells is through gradients of soluble ligands, identified by the cell-associated receptors. However, recent studies demonstrated a novel form of interaction between cancer cells and their environment through shedding of membrane exosomes, which can fuse to cells in the surrounding area and cause stromal remodeling (Al-Nedawi et al. 2008; Bhowmick et al. 2004; Skog et al. 2008). Exosomes contain microvesicles that serve as “units” of information by containing a mass of biologically active protein and RNA species, including oncogenic receptors and microRNAs (miRNAs), short noncoding RNAs that modulate gene expression (Kim et al. 2009b; Liu et al. 2009). For example, it was demonstrated that a truncated oncogenic form of EGFR, EGFRvIII, was transferred from human brain glioma cells into neighboring glioma cells deficient in EGFRvIII via secretory membrane microvesicles (exosomes) (Al-Nedawi et al. 2008). In the lungs, however, no examples of exosomal oncoprotein transfers have (yet) been described. Instead, the bulk of studies in the role of exosomes in oncogenesis have been focused in miRNAs which become deregulated in numerous cancers, including of lung origin.

Some miRNAs are notably deregulated in lung cancer. For example, low expression of let-7a miRNA and high expression of miR-155 miRNA were associated with poor clinical outcome (Yanaihara et al. 2006). At the same time, there are miRNAs overexpressed in lung cancer that have key roles in modulating cancerous cell growth and tumorigenicity such as the EGFR (Liu et al. 2010). A recent publication by Kosaka et al. (2010) reported that exosomes may be used as a means for secretion of miRNAs: miRNAs secreted from donor cells could be absorbed and remain functional in recipient cells, suggesting an innovative mechanism of intercellular communication by miRNAs release. Consistently, Pegtel et al. (2010) also demonstrated that miRNAs secreted by Epstein–Barr virus (EBV)-infected cells were transferred to and acted in uninfected recipient cells. Importantly, Kosaka et al. (2010) demonstrated that miRNA incorporation into exosomes and secretion are ceramide-/nSMase2 dependent, which underscores a novel critical role of nSMase2 in regulating exosome-dependent miRNA dispersal.

Moreover, the molecular mechanism by which a change in lipid composition drives vesicle budding was recently demonstrated by Trajkovic et al. (2008) to be regulated via nSMase2 and, thusly, ceramide. Using mass spectrometric analyses, Trajkovic et al. demonstrated that ceramide levels were increased in secreted proteolipid protein-containing exosomes purified from cell culture medium. Moreover, disrupting nSMase2 expression by RNA interference or the use of specific inhibitors reduced secretion of these exosomes, suggesting that aggregation of ceramide-enriched microdomains leads to intraluminal vesicles formation.

Additionally, miRNA-containing microvesicles could be recovered from the plasma of patients with tumors, including patients with lung cancer, thus potentially serving as prognostic biomarkers (Al-Nedawi et al. 2009; Chen et al. 2008; Mitchell et al. 2008). Therefore, ceramide-dependent exosomal secretion may drive the transfer of oncoproteins and onco-miRNAs between subset of cancer cell populations and, therefore, necessitate the

evaluation of ceramide and exosomes in the distribution oncogenes and their transforming phenotype in several cancer types, including lung cancer.

Though several groups continue to study the mechanism(s) of nSMase2 regulation in lung epithelial cells and other tissues, ceramide generation and its potential function(s) in oncogenic signaling remain poorly understood. However, within the setting of lung injury and lung cancer, recent studies by Goldkorn and colleagues (Filosto et al. 2011b) documented the proliferative effects of cigarette smoke-induced oxidative stress via the epidermal growth factor (EGF) receptor, a membrane-bound protein important for growth signaling, and possible potentiation of its activation by ceramide generation in the plasma membrane, as described in the following sections.

9 Cigarette Smoke-Induced Oxidative Stress and Its Effects on Oncogenic EGF Receptor Activation in Lung Cancer

In chronic smokers, the plasma membrane of airway epithelial cells works as the first line of defense against gaseous oxidants. Though this scenario depicts lung injury fairly well as previously discussed with ceramide, simultaneous studies on the EGF receptor (EGFR) portray the bipolar effects of cigarette smoke: Proliferative signaling becomes activated during oxidative stress in airway epithelial cells (Goldkorn et al. 2005; Ravid et al. 2002).

The EGFR is a transmembrane receptor tyrosine kinase (RTK) of the ErbB family, which includes ErbB1–4 and is the prototypical “master” regulator of cell growth in normal cells and tumorigenesis in lung cancer. The classical model of EGFR activation has been established wherein (1) binding of its extracellular ligand EGF (2) induces receptor dimerization leading to (3) activation of its intracellular tyrosine kinase (TK) domain and subsequent (4) auto-phosphorylation of specific tyrosines on the C-terminal “tail” of the receptor, ultimately (5) initiating a cascade of downstream proliferative signaling. Simultaneously, the activated EGFR is internalized in a clathrin-dependent manner and downregulated via lysosomal degradation. However, deregulation of EGFR function can happen for many reasons, including EGFR somatic mutations or its overexpression, leading to EGFR-driven lung tumorigenesis. Therefore, a collective effort by the field over the last two decades has led to better understanding the mechanism of EGFR kinase activation and its targeting by tyrosine kinase inhibitors.

Tyrosine kinase inhibitors (TKIs) are small molecules that inhibit TK activity by competitively binding to the ATP binding site of the EGFR. In the early 2000s, several TKIs—erlotinib (Tarceva™) and gefitinib (Iressa™)—were approved by the FDA (Food and Drug Administration) for the treatment of metastatic lung cancer, while several other TKIs are in preclinical trials (e.g., TKI AG1478) and currently in the drug development pipeline (Ellis et al. 2006; Gridelli et al. 2010; Watanabe et al. 2011; Wu et al. 2011). Unfortunately, despite earlier promising clinical success, these FDA-approved TKIs failed to benefit the majority of ailing lung cancer patients. Interestingly, however, encouraging responses to the TKIs were observed in a subset of non-small cell lung cancer (NSCLC) cases, typically presenting as adenocarcinomas in female never-smokers (Yatabe 2010). These responders were found to possess mutations in the EGFR tyrosine kinase domain that constitutively

activate the receptor in a ligand-independent manner, which were thought to confer a selective growth advantage that could be efficiently neutralized by the TKIs (Lynch et al. 2004; Minna et al. 2004; Paez et al. 2004; Shigematsu and Gazdar 2006; Weinstein 2002). Most common, or “classic,” among these somatic mutations are the exon 19 deletion mutation (746–750) and the exon 21 single-point substitution mutation (L858R) (Mountzios et al. 2008; Parkin et al. 2005).

However, it has been recently observed that the same EGFR mutations are not limited to adenocarcinoma from female never-smokers. Indeed, a large number (40%) of these EGFR mutations are actually found in adenocarcinoma tumor specimens from men and people who are/were smokers (D'Angelo et al. 2011). Yet, these lung cancer patient groups of former and current smokers (harboring EGFR mutations, which are expectedly to be TKI sensitive) do not benefit from the TKI treatment. Moreover, anecdotal clinical observations suggest that lung cancer patients with smoking history tend to require up to a tenfold higher baseline dose of TKI to start during treatment. This indicates that smoking could affect the tumor's response to TKI, but the mechanisms involved are still mostly elusive.

In parallel with their ceramide studies, Goldkorn et al. investigated the effects of CS-derived reactive oxidants on the structure/function of EGFR in HAE cells in both normal and transformed (NSCLC) cells (Filosto et al. 2011b, 2012b; Goldkorn et al. 2005; Goldkorn and Filosto 2010; Khan et al. 2006, 2008; Ravid et al. 2002). Since then, it has been demonstrated that both CS and oxidative stress (H₂O₂) aberrantly activate the EGFR which can be suppressed in the presence of the antioxidant glutathione or catalase (Khan et al. 2008; Ravid et al. 2002). Specifically, the pattern of tyrosine-phosphorylation sites differs from that induced by the EGF ligand (Khan et al. 2008), resulting in an activated/signaling EGFR without the physiological negative feedback of its lysosomal degradation (Khan et al. 2006, 2008; Ravid et al. 2002). Goldkorn's group also showed that this abnormal EGFR activation impairs receptor dimerization and is not targeted/inhibited by TKIs (erlotinib, gefitinib, and AG1478) (Filosto et al. 2011b, 2012b) (Fig. 3).

Thus, it was recently demonstrated that a novel active conformation of the EGFR confers tumorigenesis and resistance to current TKIs following CS and oxidative stress exposure in HAE cells (Filosto et al. 2011b, 2012b). These findings have far-reaching implications suggesting that this oxidative stress-activated EGFR cannot be inhibited by clinical tyrosine kinase inhibitors developed for therapeutic intervention in non-small cell lung cancer, the predominant lung cancer variant in the smoker population, and may explain the sparse success rate of these drugs in patients (Filosto et al. 2012b).

Therefore, it appears that a dichotomous response to cigarette smoke occurs concurrently at the cellular level in airway epithelial cells (Goldkorn and Filosto 2010). On the one hand, ceramide, a pro-apoptotic sphingolipid, is generated by the induction of nSMase2 activity, while, on the other hand, the EGFR becomes aberrantly activated and promotes proliferative signaling. To explain these observations, Goldkorn et al. proposed a novel perhaps equally insidious function for ceramide, as the link between chronic smoking-related lung injury and tumorigenesis in the airway. That role was based on the idea that the novel EGFR

conformation, which is generated under CS exposure, can be stabilized and thus become chronic only in ceramide-enriched cellular compartments, as detailed below.

10 Cigarette Smoke-Induced Ceramide-Enriched Microdomains: Mitogenic Signaling Platforms

Importantly, oxidative stress-dependent alterations of EGFR structure/function turn out to be temperature dependent and are inhibited by cholesterol uptake at the plasma membrane, suggesting a requirement for membrane alterations (Filosto et al. 2011b). Moreover, the TKI AG1478 is ineffective in quenching EGFR phosphorylation during H₂O₂-induced ox-stress in living cells while capable of inhibiting it in a crude membrane fraction of broken cells where the membrane structure was destroyed (Filosto et al. 2011b). This supports the idea that membrane fluidity/structure may be involved in either inducing or stabilizing the ox-stress-induced active conformation of EGFR.

In recent years, several studies demonstrated that sphingolipids in cell membranes are not homogenous but are sorted into specific domains mediated by interactions between sphingolipids and cholesterol (Zhang et al. 2009). In particular, the ceramide moiety of sphingomyelin binds to cholesterol via hydrophobic van der Waals interactions (Kolesnick et al. 2000). The culmination of these strong interactions and the high local concentration of sphingolipids and cholesterol lead to the formation of sphingolipids and cholesterol-enriched membrane domains known as lipid rafts. Cholesterol and some cholesterol precursors not only interact with sphingolipids in these rafts but also stabilize the structure of rafts by filling empty spaces between the bulky sphingolipids (Kolesnick et al. 2000; Stancevic and Kolesnick 2010; Xu et al. 2001; Zhang et al. 2009).

However, when ceramide becomes free from sphingomyelin, it can displace cholesterol from the rafts (Megha and London 2004). Thus, the generation of ceramide within membrane rafts may alter both their composition and structure. Furthermore, ceramide-enriched microdomains have the tendency to fuse and form larger macrodomains, also known as ceramide-enriched membrane platforms, with a diameter from a few hundred nanometers up to several micrometers (Cremesti et al. 2001). These ceramide-enriched membrane platforms have altered biophysical properties compared to the phospholipid membrane and cholesterol-enriched lipid rafts and may serve important functions in protein sorting in cells and redistribution of membrane receptors, such as the EGFR. Indeed, ceramide-enriched membrane platforms were shown to trap and to cluster receptor molecules (Cremesti et al. 2001; Grassme et al. 2001; Gulbins and Kolesnick 2003).

Given the critical role of lipid rafts in serving as signaling platforms for many membrane receptors, including the EGFR (Heering et al. 2012), alteration in membrane structure and fluidity following ceramide generation are likely to have an important effect on lung cancer cell growth. Intriguingly, cholesterol levels do not change during oxidative stress in HAE cells (Filosto et al. 2011b). Thus, Goldkorn et al. lately proposed that membrane ceramide generated as a result of exposure to ox-stress may displace cholesterol from the rafts, leading to the relocalization of EGFR to relatively rigid ceramide-enriched lipid platforms, which may stabilize aberrant EGFR signaling. Complementing this hypothesis, it was recently

demonstrated that the CS-induced oxidative stress stabilizes aberrant interactions between the activated EGFR and Src, a non-receptor tyrosine kinase that is highly overexpressed in several types of cancers (Cai et al. 2011; Ceppi et al. 2012; Chung et al. 2009; Kim et al. 2009a). Furthermore, it was reported that phosphorylated caveolin-1 (Cav-1), a known substrate of Src, is strongly bound to EGFR under ox-stress (Khan et al. 2006, 2008). Notably, others have reported that Src stably interacts with ErbB2 (Kim et al. 2005), but not with WT EGFR, because of the structural difference in their respective kinase domains, and this Src binding was shown to confer elevated transformation ability (Marcotte et al. 2009). For these reasons, it was also proposed that such membrane alterations may also be involved in the aberrant perinuclear trafficking of EGFR and Src that is observed in HAE cells during exposure to oxidative stress (Filosto et al. 2011b; Khan et al. 2008).

Supporting this hypothesis, it was recently shown that during oxidative stress, activated EGFR and activated Src co-localize within ceramide-enriched membrane regions (Filosto et al. 2011b) (Fig. 4). At early time points of ox-stress exposure (15 min), active EGFR and elevated ceramide co-localize primarily in the plasma membrane of the cells. Interestingly, later on (30 min), this co-localization is observed mainly in a perinuclear region of the cells. Consistent with this latter observation, it was demonstrated that the ox-stress-activated EGFR, unlike the EGF-stimulated receptor, is not internalized via clathrin-coated pits. Instead, it is not degraded, remains active, and is trafficked via caveolae to the perinucleus because of strong association with phosphorylated Cav-1 (Khan et al. 2006, 2008). It has been recently shown that ceramide generation increases the recruitment of Cav-1 into caveolae (Kuebler et al. 2010; Yang et al. 2010). Taken together, ceramide generation may have a role in both stabilizing the aberrantly active EGFR and controlling the trafficking of EGFR via caveolae to the cell perinucleus that has been shown to promote tumorigenesis in lung epithelial cells under oxidative stress (Khan et al. 2008).

11 Conclusions

Sphingolipids and their roles in lung cancer offer critically needed avenues for discovery of both prognostic and diagnostic biomarkers, thereby remaining a field requiring discovery. As described earlier, the best characterized sphingolipids in lung cancer research are sphingosine-1-phosphate and ceramide which traditionally have opposing roles in determining cell fate. However, there appears to be a very fine line in the distinct roles of specific sphingolipids as evidenced by recent findings that ceramide-1-phosphate has both proliferative and apoptotic effects depending on its concentration and context. Therefore, in this chapter, we summarized several years of investigation into ceramide, a canonically pro-apoptotic sphingolipid that is starting to gain momentum in cancer research for its potential roles in proliferation and cell-to-cell communication via exosomes. However, to provide context for this novel role of ceramide, we focused on cigarette smoke and its effects on airway epithelium as a model for ceramide's role in lung cancer.

Lung cancer and lung injury are common sequelae of chronic tobacco smoke exposure as it is well established by several epidemiological studies of smoker cohorts that lung cancer incidence is significantly increased in emphysema patients. Under exposure to cigarette smoke (CS) and oxidative stress, lung injury (apoptosis) and repair (proliferation) proceed

simultaneously. Therefore, both “facets,” apoptosis and proliferation, of the coin that underlie CS-mediated lung injury and lung cancer should be addressed together.

The studies presented herein clearly establish that these dichotomous signaling pathways are indeed activated under CS exposure. On the one hand, ceramide is generated by the activation of nSMase2 during CS-induced oxidative stress, eventually leading to apoptosis which is the dominant pathway in lung injury and COPD. On the other hand, the same stimulus aberrantly activates the EGFR, leading to prolonged proliferative signaling as observed in non-small cell lung cancer. Interestingly, recent studies provide evidence that these two CS-activated pathways may actually converge and interact. Specifically, Goldkorn et al. found that EGFR and Src are preferentially co-localized in ceramide-enriched regions of the plasma membrane under CS exposure, suggesting that nSMase2/ceramide may directly affect the aberrant EGFR/Src activation and mutual interaction, leading to enhanced tumorigenic signaling. Moreover, new findings suggest that CS exposure may induce resistance to EGFR-targeted therapy, the tyrosine kinase inhibitors (TKIs), solely through posttranslational molecular changes. These molecular alterations consist of an aberrant EGFR phosphorylation pattern accompanied by an aberrant conformation and the binding to Src during CS exposure. Furthermore, at the molecular level, these observed structural anomalies of the CS-activated EGFR are potentiated by excess ceramide and ceramide-enriched microdomains generated by the CS-activated nSMase2 in the plasma membrane of lung epithelial cells.

In summary, regard for sphingolipids in human health and disease has grown over the years. Despite the ongoing evolution of the field in cancer biology as presented here, there is an urgent and great need for investigation into the role of sphingolipids in lung cancer due to the plethora of promising observations reported. Therefore, we, the authors, hope that you, the readers, are convinced of the importance of sphingolipids in lung cancer.

References

- Alberg AJ, Samet JM. Epidemiology of lung cancer. *Chest*. 2003; 123:21S–49S. [PubMed: 12527563]
- Al-Nedawi K, Meehan B, Micallef J, Lhotak V, May L, Guha A, et al. Intercellular transfer of the oncogenic receptor EGFRvIII by microvesicles derived from tumour cells. *Nat Cell Biol*. 2008; 10:619–624. [PubMed: 18425114]
- Al-Nedawi K, Meehan B, Rak J. Microvesicles: messengers and mediators of tumor progression. *Cell Cycle*. 2009; 8:2014–2018. [PubMed: 19535896]
- Bhowmick NA, Neilson EG, Moses HL. Stromal fibroblasts in cancer initiation and progression. *Nature*. 2004; 432:332–337. [PubMed: 15549095]
- Caballero A, Torres-Duque CA, Jaramillo C, Bolivar F, Sanabria F, Osorio P, et al. Prevalence of COPD in five Colombian cities situated at low, medium, and high altitude (PREPOCOL study). *Chest*. 2008; 133:343–349. [PubMed: 17951621]
- Cai H, Babic I, Wei X, Huang J, Witte ON. Invasive prostate carcinoma driven by c-Src and androgen receptor synergy. *Cancer Res*. 2011; 71:862–872. [PubMed: 21135112]
- Ceppi P, Rapa I, Lo Iacono M, Righi L, Giorcelli J, Pautasso M, et al. Expression and pharmacological inhibition of thymidylate synthase and Src kinase in nonsmall cell lung cancer. *Int J Cancer*. 2012; 130:1777–1786. [PubMed: 21618517]
- Chan C, Goldkorn T. Ceramide path in human lung cell death. *Am J Respir Cell Mol Biol*. 2000; 22:460–468. [PubMed: 10745027]

- Chen X, Ba Y, Ma L, Cai X, Yin Y, Wang K, et al. Characterization of microRNAs in serum: a novel class of biomarkers for diagnosis of cancer and other diseases. *Cell Res.* 2008; 18:997–1006. [PubMed: 18766170]
- Chung BM, Dimri M, George M, Reddi AL, Chen G, Band V, et al. The role of cooperativity with Src in oncogenic transformation mediated by non-small cell lung cancer-associated EGF receptor mutants. *Oncogene.* 2009; 28:1821–1832. [PubMed: 19305428]
- Church DF, Pryor WA. Free-radical chemistry of cigarette smoke and its toxicological implications. *Environ Health Perspect.* 1985; 64:111–126. [PubMed: 3007083]
- Clarke CJ, Guthrie JM, Hannun YA. Regulation of neutral sphingomyelinase-2 (nSMase2) by tumor necrosis factor- α involves protein kinase C- δ in lung epithelial cells. *Mol Pharmacol.* 2008; 74:1022–1032. [PubMed: 18653803]
- Cremesti A, Paris F, Grassme H, Holler N, Tschopp J, Fuks Z, et al. Ceramide enables fas to cap and kill. *J Biol Chem.* 2001; 276:23954–23961. [PubMed: 11287428]
- D'Angelo SP, Pietanza MC, Johnson ML, Riely GJ, Miller VA, Sima CS, et al. Incidence of EGFR exon 19 deletions and L858R in tumor specimens from men and cigarette smokers with lung adenocarcinomas. *J Clin Oncol.* 2011; 29:2066–2070. [PubMed: 21482987]
- de Molina AR, de la Cueva A, Machado-Pinilla R, Rodriguez-Fanjul V, Del Pulgar TG, Cebrian A, et al. Acid ceramidase as a chemotherapeutic target to overcome resistance to the antitumoral effect of choline kinase α inhibition. *Curr Cancer Drug Targets.* 2012; 12(6):617–24. [PubMed: 22515519]
- de Torres JP, Bastarrika G, Wisnivesky JP, Alcaide AB, Campo A, Seijo LM, et al. Assessing the relationship between lung cancer risk and emphysema detected on low-dose CT of the chest. *Chest.* 2007; 132:1932–1938. [PubMed: 18079226]
- Devillard R, Galvani S, Thiers JC, Guenet JL, Hannun Y, Bielawski J, et al. Stress-induced sphingolipid signaling: role of type-2 neutral sphingomyelinase in murine cell apoptosis and proliferation. *PLoS One.* 2010; 5:e9826. [PubMed: 20352118]
- Ellis AG, Doherty MM, Walker F, Weinstock J, Nerrie M, Vitali A, et al. Preclinical analysis of the analinoquinazoline AG1478, a specific small molecule inhibitor of EGF receptor tyrosine kinase. *Biochem Pharmacol.* 2006; 71:1422–1434. [PubMed: 16522318]
- Esme H, Cemek M, Sezer M, Saglam H, Demir A, Melek H, et al. High levels of oxidative stress in patients with advanced lung cancer. *Respirology.* 2008; 13:112–116. [PubMed: 18197920]
- Filosto S, Fry W, Knowlton AA, Goldkorn T. Neutral sphingomyelinase 2 (nSMase2) is a phosphoprotein regulated by calcineurin (PP2B). *J Biol Chem.* 2010; 285:10213–10222. [PubMed: 20106976]
- Filosto S, Castillo S, Danielson A, Franzi L, Khan E, Kenyon N, et al. Neutral sphingomyelinase 2: a novel target in cigarette smoke-induced apoptosis and lung injury. *Am J Respir Cell Mol Biol.* 2011a; 44:350–360. [PubMed: 20448054]
- Filosto S, Khan E, Tognon E, Becker C, Ashfaq M, Ravid T, et al. EGF receptor exposed to oxidative stress acquires abnormal phosphorylation and aberrant activated conformation that impairs canonical dimerization. *PLoS One.* 2011b; 6:e23240. [PubMed: 21853092]
- Filosto S, Ashfaq M, Chung S, Fry W, Goldkorn T. Neutral sphingomyelinase 2 activity and protein stability are modulated by phosphorylation of five conserved serines. *J Biol Chem.* 2012a; 287:514–522. [PubMed: 22074919]
- Filosto S, Becker CR, Goldkorn T. Cigarette smoke induces aberrant EGF receptor activation that mediates lung cancer development and resistance to tyrosine kinase inhibitors. *Mol Cancer Ther.* 2012b; 11:795–804. [PubMed: 22302097]
- Foley TD, Petro LA, Stredny CM, Coppa TM. Oxidative inhibition of protein phosphatase 2A activity: role of catalytic subunit disulfides. *Neurochem Res.* 2007; 32:1957–1964. [PubMed: 17562162]
- Goldkorn T, Filosto S. Lung injury and cancer: mechanistic insights into ceramide and EGFR signaling under cigarette smoke. *Am J Respir Cell Mol Biol.* 2010; 43:259–268. [PubMed: 20525802]
- Goldkorn T, Balaban N, Shannon M, Chea V, Matsukuma K, Gilchrist D, et al. H₂O₂ acts on cellular membranes to generate ceramide signaling and initiate apoptosis in tracheobronchial epithelial cells. *J Cell Sci.* 1998; 111:3209–3220. [PubMed: 9763515]

- Goldkorn T, Ravid T, Khan EM. Life and death decisions: ceramide generation and EGF receptor trafficking are modulated by oxidative stress. *Antioxid Redox Signal*. 2005; 7:119–128. [PubMed: 15650401]
- Gomez-Munoz A, Kong JY, Salh B, Steinbrecher UP. Ceramide-1-phosphate blocks apoptosis through inhibition of acid sphingomyelinase in macrophages. *J Lipid Res*. 2004; 45:99–105. [PubMed: 14523050]
- Grassme H, Jekle A, Riehle A, Schwarz H, Berger J, Sandhoff K, et al. CD95 signaling via ceramide-rich membrane rafts. *J Biol Chem*. 2001; 276:20589–20596. [PubMed: 11279185]
- Gridelli C, Maione P, Bareschino MA, Schettino C, Sacco PC, Ambrosio R, et al. Erlotinib in the treatment of non-small cell lung cancer: current status and future developments. *Anticancer Res*. 2010; 30:1301–1310. [PubMed: 20530444]
- Gulbins E, Kolesnick R. Raft ceramide in molecular medicine. *Oncogene*. 2003; 22:7070–7077. [PubMed: 14557812]
- Hannun YA, Obeid LM. Many ceramides. *J Biol Chem*. 2011; 286:27855–27862. [PubMed: 21693702]
- Heering J, Weis N, Holeiter M, Neugart F, Staebler A, Fehm TN, et al. Loss of the ceramide transfer protein augments EGF receptor signaling in breast cancer. *Cancer Res*. 2012; 72(11):2855–66. [PubMed: 22472120]
- Inokuchi J, Jimbo M, Momosaki K, Shimeno H, Nagamatsu A, Radin NS. Inhibition of experimental metastasis of murine Lewis lung carcinoma by an inhibitor of glucosylceramide synthase and its possible mechanism of action. *Cancer Res*. 1990; 50:6731–6737. [PubMed: 2145065]
- Khan E, Heidinger J, Levy M, Lisanti M, Ravid T, Goldkorn T. EGF receptor exposed to oxidative stress undergoes Src- and caveolin-1-dependent perinuclear trafficking. *J Biol Chem*. 2006; 281:14486–14493. [PubMed: 16407214]
- Khan EM, Lanir R, Danielson AR, Goldkorn T. EGF receptor exposed to cigarette smoke is aberrantly activated and undergoes perinuclear trafficking. *FASEB J*. 2008; 22:910–917. [PubMed: 17971399]
- Kim H, Chan R, Dankort DL, Zuo D, Najoukas M, Park M, et al. The c-Src tyrosine kinase associates with the catalytic domain of ErbB-2: implications for ErbB-2 mediated signaling and transformation. *Oncogene*. 2005; 24:7599–7607. [PubMed: 16170374]
- Kim LC, Song L, Haura EB. Src kinases as therapeutic targets for cancer. *Nat Rev Clin Oncol*. 2009a; 6:587–595. [PubMed: 19787002]
- Kim VN, Han J, Siomi MC. Biogenesis of small RNAs in animals. *Nat Rev Mol Cell Biol*. 2009b; 10:126–139. [PubMed: 19165215]
- Kolesnick RN, Goni FM, Alonso A. Compartmentalization of ceramide signaling: physical foundations and biological effects. *J Cell Physiol*. 2000; 184:285–300. [PubMed: 10911359]
- Kosaka N, Iguchi H, Yoshioka Y, Takeshita F, Matsuki Y, Ochiya T. Secretory mechanisms and intercellular transfer of microRNAs in living cells. *J Biol Chem*. 2010; 285:17442–17452. [PubMed: 20353945]
- Kuebler WM, Yang Y, Samapati R, Uhlig S. Vascular barrier regulation by PAF, ceramide, caveolae, and NO - an intricate signaling network with discrepant effects in the pulmonary and systemic vasculature. *Cell Physiol Biochem*. 2010; 26:29–40. [PubMed: 20502002]
- Lavrentiadou SN, Chan C, Ravid T, Tsaba A, van der Vliet A, Rasooly R, et al. Ceramide-mediated apoptosis in lung epithelial cells is regulated by GSH. *Am J Respir Cell Mol Biol*. 2001; 25:676–684. [PubMed: 11726392]
- Levy M, Castillo SS, Goldkorn T. nSMase2 activation and trafficking are modulated by oxidative stress to induce apoptosis. *Biochem Biophys Res Commun*. 2006; 344:900–905. [PubMed: 16631623]
- Levy M, Khan E, Careaga M, Goldkorn T. Neutral sphingomyelinase 2 is activated by cigarette smoke to augment ceramide-induced apoptosis in lung cell death. *Am J Physiol Lung Cell Mol Physiol*. 2009; 297:125–133.
- Liu X, Sempere LF, Galimberti F, Freemantle SJ, Black C, Dragnev KH, et al. Uncovering growth-suppressive MicroRNAs in lung cancer. *Clin Cancer Res*. 2009; 15:1177–1183. [PubMed: 19228723]

- Liu X, Sempere LF, Ouyang H, Memoli VA, Andrew AS, Luo Y, et al. MicroRNA-31 functions as an oncogenic microRNA in mouse and human lung cancer cells by repressing specific tumor suppressors. *J Clin Invest*. 2010; 120:1298–1309. [PubMed: 20237410]
- Lynch TJ, Bell DW, Sordella R, Gurubhagavatula S, Okimoto RA, Brannigan BW, et al. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. *N Engl J Med*. 2004; 350:2129–2139. [PubMed: 15118073]
- Maceyka M, Harikumar KB, Milstien S, Spiegel S. Sphingosine-1-phosphate signaling and its role in disease. *Trends Cell Biol*. 2012; 22:50–60. [PubMed: 22001186]
- Marcotte R, Zhou L, Kim H, Roskelley CD, Muller WJ. c-Src associates with ErbB2 through an interaction between catalytic domains and confers enhanced transforming potential. *Mol Cell Biol*. 2009; 29:5858–5871. [PubMed: 19704002]
- Megha, London E. Ceramide selectively displaces cholesterol from ordered lipid domains (rafts): implications for lipid raft structure and function. *J Biol Chem*. 2004; 279:9997–10004. [PubMed: 14699154]
- Merrill AH Jr. Sphingolipid and glycosphingolipid metabolic pathways in the era of sphingolipidomics. *Chem Rev*. 2011; 111:6387–6422. [PubMed: 21942574]
- Minna JD, Gazdar AF, Sprang SR, Herz J. Cancer. A bull's eye for targeted lung cancer therapy. *Science*. 2004; 304:1458–1461. [PubMed: 15178790]
- Mitchell PS, Parkin RK, Kroh EM, Fritz BR, Wyman SK, Pogosova-Agadjanyan EL, et al. Circulating microRNAs as stable blood-based markers for cancer detection. *Proc Natl Acad Sci USA*. 2008; 105:10513–10518. [PubMed: 18663219]
- Mitra P, Maceyka M, Payne SG, Lamour N, Milstien S, Chalfant CE, et al. Ceramide kinase regulates growth and survival of A549 human lung adenocarcinoma cells. *FEBS Lett*. 2007; 581:735–740. [PubMed: 17274985]
- Morales A, Lee H, Goni FM, Kolesnick R, Fernandez-Checa JC. Sphingolipids and cell death. *Apoptosis*. 2007; 12:923–939. [PubMed: 17294080]
- Mountzios G, Fouret P, Soria JC. Mechanisms of disease: signal transduction in lung carcinogenesis – a comparison of smokers and never-smokers. *Nat Clin Pract Oncol*. 2008; 5:610–618. [PubMed: 18628738]
- Mukhopadhyay A, Saddoughi SA, Song P, Sultan I, Ponnusamy S, Senkal CE, et al. Direct interaction between the inhibitor 2 and ceramide via sphingolipid-protein binding is involved in the regulation of protein phosphatase 2A activity and signaling. *FASEB J*. 2009; 23:751–763. [PubMed: 19028839]
- Mullen TD, Spassieva S, Jenkins RW, Kitatani K, Bielawski J, Hannun YA, et al. Selective knockdown of ceramide synthases reveals complex interregulation of sphingolipid metabolism. *J Lipid Res*. 2011; 52:68–77. [PubMed: 20940143]
- Nikolova-Karakashian M, Karakashian A, Rutkute K. Role of neutral sphingomyelinases in aging and inflammation. *Subcell Biochem*. 2008; 49:469–486. [PubMed: 18751923]
- Nowak D, Antczak A, Krol M, Pietras T, Shariati B, Bialasiewicz P, et al. Increased content of H₂O₂ in the expired breath of cigarette smokers. *Eur Respir J*. 1996; 9:652–657. [PubMed: 8726926]
- Ogretmen B, Schady D, Usta J, Wood R, Kravaka JM, Luberto C, et al. Role of ceramide in mediating the inhibition of telomerase activity in A549 human lung adenocarcinoma cells. *J Biol Chem*. 2001; 276:24901–24910. [PubMed: 11335714]
- Oskouian B, Saba JD. Cancer treatment strategies targeting sphingolipid metabolism. *Adv Exp Med Biol*. 2010; 688:185–205. [PubMed: 20919655]
- Paez JG, Janne PA, Lee JC, Tracy S, Greulich H, Gabriel S, et al. EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. *Science*. 2004; 304:1497–1500. [PubMed: 15118125]
- Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. *CA Cancer J Clin*. 2005; 55:74–108. [PubMed: 15761078]
- Pegtel DM, Cosmopoulos K, Thorley-Lawson DA, van Eijndhoven MA, Hopmans ES, Lindenberg JL, et al. Functional delivery of viral miRNAs via exosomes. *Proc Natl Acad Sci USA*. 2010; 107:6328–6333. [PubMed: 20304794]

- Ponnusamy S, Meyers-Needham M, Senkal CE, Saddoughi SA, Sentelle D, Selvam SP, et al. Sphingolipids and cancer: ceramide and sphingosine-1-phosphate in the regulation of cell death and drug resistance. *Future Oncol.* 2010; 6:1603–1624. [PubMed: 21062159]
- Ravid T, Sweeney C, Gee P, Carraway KR, Goldkorn T. EGF receptor activation under oxidative stress fails to promote c-Cbl mediated down regulation. *J Biol Chem.* 2002; 12:12.
- Ravid T, Tsaba A, Gee P, Rasooly R, Medina EA, Goldkorn T. Ceramide accumulation precedes caspase-3 activation during apoptosis of A549 human lung adeno. cells. *Am J Physiol Lung Cell Mol Physiol.* 2003; 284:L1082–1092. [PubMed: 12576296]
- Rutkute K, Asmis RH, Nikolova-Karakashian MN. Regulation of neutral sphingomyelinase-2 by GSH: a new insight to the role of oxidative stress in aging-associated inflammation. *J Lipid Res.* 2007a; 48:2443–2452. [PubMed: 17693623]
- Rutkute K, Karakashian AA, Giltaiy NV, Dobierzewska A, Nikolova-Karakashian MN. Aging in rat causes hepatic hyperresponsiveness to IL-1 β which is mediated by nSMase2. *Hepatology.* 2007b; 46:1166–1176. [PubMed: 17668873]
- Schnitzer SE, Weigert A, Zhou J, Brune B. Hypoxia enhances sphingosine kinase 2 activity and provokes sphingosine-1-phosphate-mediated chemoresistance in A549 lung cancer cells. *Mol Cancer Res.* 2009; 7:393–401. [PubMed: 19240180]
- Shigematsu H, Gazdar AF. Somatic mutations of epidermal growth factor receptor signaling pathway in lung cancers. *Int J Cancer.* 2006; 118:257–262. [PubMed: 16231326]
- Skog J, Wurdinger T, van Rijn S, Meijer DH, Gainche L, Sena-Esteves M, et al. Glioblastoma microvesicles transport RNA and proteins that promote tumour growth and provide diagnostic biomarkers. *Nat Cell Biol.* 2008; 10:1470–1476. [PubMed: 19011622]
- Stancevic B, Kolesnick R. Ceramide-rich platforms in transmembrane signaling. *FEBS Lett.* 2010; 584:1728–1740. [PubMed: 20178791]
- Sznajder JJ, Fraiman A, Hall JB, Sanders W, Schmidt G, Crawford G, et al. Increased H₂O₂ in the expired breath of patients with acute hypoxemic respiratory failure. *Chest.* 1989; 96:606–612. [PubMed: 2766820]
- Trajkovic K, Hsu C, Chiantia S, Rajendran L, Wenzel D, Wieland F, et al. Ceramide triggers budding of exosome vesicles into multivesicular endosomes. *Science.* 2008; 319:1244–1247. [PubMed: 18309083]
- Uhlig S, Gulbins E. Sphingolipids in the lungs. *Am J Respir Crit Care Med.* 2008; 178:1100–1114. [PubMed: 18755926]
- Watanabe S, Tanaka J, Ota T, Kondo R, Tanaka H, Kagamu H, et al. Clinical responses to EGFR-tyrosine kinase inhibitor retreatment in non-small cell lung cancer patients who benefited from prior effective gefitinib therapy: a retrospective analysis. *BMC Cancer.* 2011; 11:1. [PubMed: 21194487]
- Weinstein IB. Cancer. Addiction to oncogenes—the Achilles heel of cancer. *Science.* 2002; 297:63–64. [PubMed: 12098689]
- Williams MD, Chance B. Spontaneous chemiluminescence of human breath. Spectrum, lifetime, temporal distribution, and correlation with peroxide. *J Biol Chem.* 1983; 258:3628–3631. [PubMed: 6833221]
- Wu BX, Clarke CJ, Hannun YA. Mammalian neutral sphingomyelinases: regulation and roles in cell signaling responses. *Neuromolecular Med.* 2010; 12:320–330. [PubMed: 20552297]
- Wu JY, Wu SG, Yang CH, Chang YL, Chang YC, Hsu YC, et al. Comparison of gefitinib and erlotinib in advanced NSCLC and the effect of EGFR mutations. *Lung Cancer.* 2011; 72:205–212. [PubMed: 20832137]
- Xu X, Bittman R, Duportail G, Heissler D, Vilcheze C, London E. Effect of the structure of natural sterols and sphingolipids on the formation of ordered sphingolipid/sterol domains (rafts). Comparison of cholesterol to plant, fungal, and disease-associated sterols and comparison of sphingomyelin, cerebrosides, and ceramide. *J Biol Chem.* 2001; 276:33540–33546. [PubMed: 11432870]
- Yamada T, Bando H, Takeuchi S, Kita K, Li Q, Wang W, et al. Genetically engineered humanized anti-ganglioside GM2 antibody against multiple organ metastasis produced by GM2-expressing small-cell lung cancer cells. *Cancer Sci.* 2011; 102:2157–2163. [PubMed: 21895875]

- Yanaihara N, Caplen N, Bowman E, Seike M, Kumamoto K, Yi M, et al. Unique microRNA molecular profiles in lung cancer diagnosis and prognosis. *Cancer Cell*. 2006; 9:189–198. [PubMed: 16530703]
- Yang Y, Yin J, Baumgartner W, Samapati R, Solymosi EA, Reppien E, et al. Platelet-activating factor reduces endothelial nitric oxide production: role of acid sphingomyelinase. *Eur Respir J*. 2010; 36:417–427. [PubMed: 19926744]
- Yatabe Y. EGFR mutations and the terminal respiratory unit. *Cancer Metastasis Rev*. 2010; 29:23–36. [PubMed: 20135199]
- Zhang Y, Li X, Becker KA, Gulbins E. Ceramide-enriched membrane domains-structure and function. *Biochim Biophys Acta*. 2009; 1788:178–183. [PubMed: 18786504]

Figure 1.

Cigarette smoke (CS)-induced oxidative stress concomitantly generates ceramide which mediates apoptotic signaling and activates the epidermal growth factor receptor (EGFR) which mediates proliferative signaling. Though CS-induced lung injury is initially dominant, epidemiological studies suggest smoking-related lung cancer becomes promoted in this environment

Figure 2.

Cigarette smoke (CS)-induced oxidative stress activates the neutral sphingomyelinase 2 (nSMase2) in human airway epithelial cells via phosphorylation of its serine residues, leading to ceramide accumulation in the inner leaflet of the plasma membrane

Figure 3.

Cigarette smoke (CS)-induced oxidative stress generates an aberrantly phosphorylated EGFR with a noncanonical activated conformation. This CS-activated EGFR is not readily degraded and has prolonged proliferation signaling and is “resistant” to tyrosine kinase inhibitors (TKIs) used clinically as therapy for EGFR-driven lung cancers

Figure 4. Cigarette smoke (CS)-induced oxidative stress activates neutral sphingomyelinase 2 (nSMase2), leading to ceramide accumulation and subsequent formation of ceramide-enriched microdomains in the plasma membrane. Within these ceramide-enriched regions, the aberrantly activated EGFR is stabilized and promotes tumorigenesis in lung epithelial cells