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Forum Editorial

Life and Death Decisions: Ceramide Generation and EGF Receptor Trafficking Are Modulated by Oxidative Stress

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ABSTRACT

Reactive oxidants are associated with the pathogenesis of pulmonary diseases and affect various cell functions, from proliferation to apoptosis. We have shown that oxidants exert growth control on airway epithelial cells by modulating upstream receptor function. Additionally, hydrogen peroxide-mediated oxidative stress modulates ceramide levels to induce apoptosis in lung epithelium. Depletion of glutathione in lung epithelial cells results in ceramide accumulation, suggesting that ceramide elevation, coupled to oxidative stress, initiates apoptosis. While it is desirable to prevent cell death and tissue injury induced by oxidants in diseases such as asthma or acute respiratory distress syndrome, the opposite is sought in cancer. But oxidants may also activate growth factor receptors, enhancing cell proliferation and facilitating tumor promotion. Under oxidative stress, phosphorylation of the epidermal growth factor receptor (EGFR) is abrogated at tyrosine 1,045, the docking site for the ubiquitin ligase c-Cbl, rendering EGFR unable to recruit c-Cbl and be ubiquitylated and degraded. We thus proposed that this deficiency, which confers prolonged receptor signaling at the plasma membrane, links oxidative stress, EGFR, and tumorigenesis. Decoding the molecular interactions between oxidative stress and ceramide pathways and characterizing ubiquitylation control of receptor desensitization should provide new strategies for intervention in diverse pulmonary diseases and in diagnosing and eradicating cancer. *Antioxid. Redox Signal.* 7, 119–128.

INTRODUCTION

Reactive oxygen species (ROS), a group of ubiquitous molecules that include species such as superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radicals ($^{\bullet}OH$), regulate important steps in the signal transduction cascades and many critical cellular events (57). As a result, ROS are involved in biologic processes ranging from normal tissue homeostasis to many human diseases. At first, the participation of ROS in disease was explained with simplistic chemistry in which critical cell proteins and lipids were randomly oxidized and rendered inactive for their roles in normal cell function (30). The recognition that ROS function as signaling molecules has been more recent.

In the present article, we extend our earlier studies, which investigated the mechanism of H_2O_2-induced apoptotic pathway by ceramide generation (7, 23, 42) and examine the possible role of ROS in cell proliferation through epidermal growth factor (EGF) receptor (EGFR) signaling (24, 64). The purpose of this article is to provide the perspective upon which to consider the mechanistic studies of ROS modulation of both cell proliferation and apoptosis (Fig. 1).

Several recent studies describe roles for ROS in the pathogenesis of pulmonary diseases such as acute respiratory distress syndrome (ARDS), chronic obstructive pulmonary disease, asthma, and interstitial pulmonary fibrosis. In the lung, epithelial cells of the airway and the alveolar compartments are constantly exposed to airborne environmental stressors. Their ability to adapt to injury from these insults is necessary in maintaining lung function. One response to cellular injury is to give up the fight and die to permit neighboring cells to replicate and replace the injured cell. Another response, which

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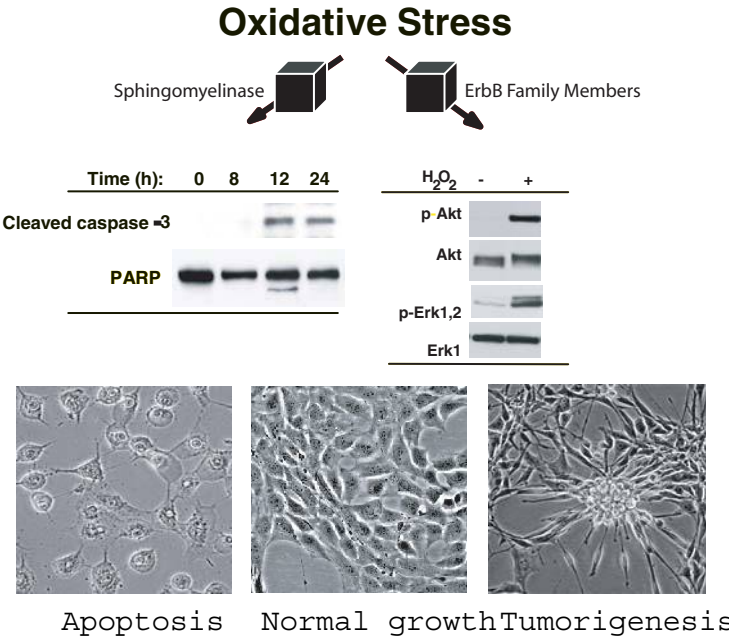

FIG. 1. Oxidative stress modulates both cell proliferation and apoptosis. (**A**) A549 cells were exposed to 150 μ*M* H_2O_2 for 1 h followed by incubation for the indicated time points with media containing 10% fetal bovine serum. Then cells were lysed in Triton X buffer [1% Triton X-100, 5 m*M* EDTA, 5 m*M* EGTA, and protease inhibitor cocktail (Sigma) in phosphate-buffered saline], and proteins were analyzed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotted using anti-poly (ADP-ribose) polymerase (anti-PARP; BD Pharmingen, San Diego, CA, U.S.A.) and anti-cleaved caspase-3 (Cell Signaling Technology, Beverly, MA, U.S.A.) antibodies. (**B**) A549 cells were exposed to 1 U/ml glucose oxidase (to generate H_2O_2) for 15 min. and then lysed [with buffer containing 50 m*M* Tris, pH 7.5, 150 m*M* NaCl, 10% glycerol, 1% Igepal, 1 m*M* EGTA, protease inhibitor cocktail (Sigma), and phosphatase inhibitor cocktail (Sigma)]. The lysates were subjected to SDS-PAGE and immunoblotted with the indicated antibodies from Cell Signaling Technology.

involves activation of EGFR by oxidants, has recently been shown to coincide with enhanced cell proliferation (65) and to facilitate tumor promotion processes (35).

Our recent studies provide a direct link between the two important aspects of mammalian stress responses. On the one hand, the generation of ROS is coupled to the activation of the sphingomyelin/ceramide cycle leading to apoptosis (23), and on the other hand, exposure to H_2O_2-induced oxidative stress preferentially enhances tyrosine phosphorylation of the EGFR, thereby generating an aberrantly phosphorylated receptor, which may promote cell hyperplasia (24). Yet the molecular mechanisms that link oxidative stress with the ceramide cycle leading to apoptosis, or with EGFR signaling and tumorigenic responses, are still being extensively investigated.

ROS AND APOPTOSIS

So far, there are opposing reports as to whether reactive oxidants inhibit or promote apoptosis. We activated the death pathway in primary tracheobronchial epithelial (TBE) cells with H_2O_2 (20–200 μ*M*) and observed the morphological changes and DNA fragmentation associated with apoptosis (23). We found that H_2O_2 acted directly on TBE cell membrane preparations devoid of nuclei, stimulating sphingomyelin hydrolysis to ceramide through a neutral Mg^{2+}-dependent sphingomyelinase (nSMase), and demonstrated that protein kinase counteracted ceramide-mediated apoptosis in the epithelium of the lung (7). Then Lavrentiadou *et al.* (42) demonstrated that either administration of exogenous H_2O_2 or enhancement of endogenously generated H_2O_2 (by administration of aminotriazole) was effective in depleting cellular glutathione (GSH) and initiating ceramide-induced apoptosis. Both scenarios are relevant to lung epithelium. H_2O_2 is a ubiquitous molecule and able to cross cell membranes freely. It is present in several air pollutants, including the vapor phase of cigarette smoke. It is also detected in exhaled air of humans (71), and amounts of exhaled H_2O_2 appear greater in subjects with lung inflammation (60) and in cigarette smokers (47). However, the key intracellular sites of ROS generation that contribute to apoptosis in lung epithelium are still unknown. Evidently, the mitochondrial electron transport chain is a critical source of ROS in apoptosis. Recent studies show that ceramide exerts a direct effect on mitochondria leading to production of H_2O_2 by inhibition of electron flow at the ubiquinone pool of complex III (21). However, apoptosis is a highly complex biochemical process, and the increase in mitochondrial ROS generation may occur rather late in the route. Growing evidence supports the view that an early burst of ROS generation may also be involved in starting the apoptosis pathway in various models. Lavrentiadou and colleagues (42) demonstrated this by the administration of aminotriazole, a specific inhibitor of catalase, which led to rapid accumulation of ceramide, a proximal event in the apoptosis model.

GSH MODULATES CERAMIDE GENERATION AND APOPTOSIS

The lung is continuously exposed to oxidants and is therefore armed with antioxidants. Both lung epithelial cells and the epithelial lining fluid (ELF) have relatively high concentrations of GSH, the main antioxidant in the lung epithelium. Lavrentiadou *et al.* (42) showed that low GSH levels were required for ceramide production, whereas high GSH levels inhibit the generation of ceramide. The decreased levels of GSH and increased levels of ceramide correlate with the induction

of apoptosis in A549 lung epithelial cells. Moreover, GSH and *N*-acetylcysteine, but not other thiol-containing antioxidants or oxidized glutathione (GSSG), were shown to inhibit H_2O_2-mediated induction of ceramide and apoptosis. Therefore, GSH plays a critical role in preventing lung epithelial cell apoptosis. In this model, inhibitory effects on ceramide production were observed with both extracellular and intracellular GSH. The effects of extracellular GSH are primarily applicable to lung epithelium. It is interesting that even a short exposure of cells to 250 μ*M* H_2O_2 for 1 h, followed by growth in regular medium, is sufficient to induce apoptosis (42). This demonstrates that the events that control the fate of the cells occur within this hour, during which GSH is depleted and ceramide is generated. Lavrentiadou *et al.* (42) proposed that in lung epithelial cells, the membrane-bound nSMase may exist as an inactive form inhibited by high levels of both intra- and extracellular GSH present in ELF, thus maintaining low levels of ceramide (Fig. 2). The inhibition of nSMase may render lung cells less sensitive and less susceptible to oxidants, to which they are ordinarily exposed. This would increase the threshold for ceramide elevation required for the induction of apoptosis. However, once oxidant levels increase, they decrease GSH levels, thereby overcoming its inhibitory effect on nSMase. Therefore, ceramide is elevated and the apoptotic pathway is initiated.

NITRIC OXIDE (NO) MODULATION OF CERAMIDE GENERATION AND APOPTOSIS

Numerous reports have confirmed the ability of NO to start apoptosis (5, 6). However, not all studies addressed whether endogenously generated NO would be sufficient to induce the death program. The formation of both NO and O_2^-; is frequently linked with cell death. In particular, the reaction product of this radical–radical contact, peroxynitrite ($ONOO^-$), is very reactive and is believed to be partially responsible for NO toxicity. However, when cells were exposed to NO donors and O_2^- generating systems, which allowed the continuous generation of both radicals over an extended period of time, the generation of both radicals was nondestructive (in macrophages, chondrocytes, and mesangial cells), whereas the generation of either NO or O_2^- induced apoptosis in the same cells (5, 6), which may provide some explanation for the proposed protective function of NO during ischemia–reperfusion or myocardial injury (5, 6).

NO elevates ceramide levels in the cell by a dual mechanism: it stimulates the ceramide-generating enzymes, acidic and neutral sphingomyelinases, thereby leading to an increase in ceramide production, as well as inhibits the ceramide-degrading enzymes, acidic and neutral ceramidases, resulting in an enhanced elevation of ceramide steady-state levels (36, 37). In some cells (*e.g.*, endothelial), costimulation with NO and O_2^-, which leads to the generation of $ONOO^-$, causes a synergistic augmentation of ceramide production and apoptosis. On the contrary, costimulation of mesenchymal cells with NO and O_2^- neutralized both NO-induced ceramide generation and apoptosis, even though O_2^- alone induced ceramide generation and apoptosis (48). Therefore, it is possible that the ratio of NO and O_2^- may determine whether these cells live or die.

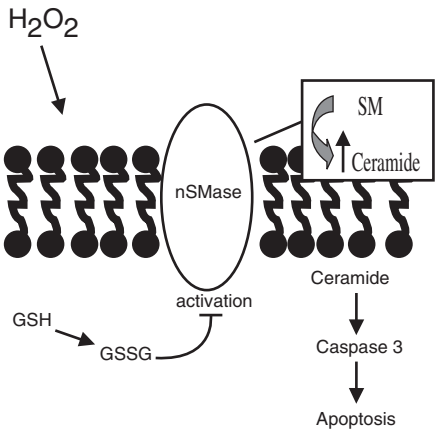

FIG. 2. The role of oxidants (H_2O_2) and anti-oxidants (GSH) in ceramide generation and apoptosis. GSH inhibits nSMase activity, thus maintaining low ceramide levels. Stress factors (*i.e.*, exposure to H_2O_2) result in ROS-mediated depletion of GSH, which could induce apoptosis through activation of nSMase and ceramide generation. This may be a critical event in the induction of apoptosis by activation of nSMase and generation of ceramide.

CERAMIDE ACCUMULATION, CASPASE ACTIVATION, AND Bcl-2 INHIBITION OF APOPTOSIS

The antiapoptotic activity of Bcl-2 has been thought to be due to its ability to inhibit caspase-3 activity (51). The role of Bcl-2 overexpression in modulating the ceramide pathway, however, is controversial. For example, Tepper *et al.* (62) showed that Bcl-2 overexpression reduced CD95-induced ceramide accumulation in Jurkat cells stably transfected with the human Bcl-2 cDNA, whereas Jaffrezou *et al.* (39) demonstrated that Bcl-2 had no effect on ceramide generation induced by C_6-ceramide in the myeloid cell line HL60/Bcl-2, genetically engineered to overexpress Bcl-2. Only a few reports demonstrate that Bcl-2 regulates ceramide formation (54, 74), whereas most studies indicate that Bcl-2 blocks apoptosis, but does not affect ceramide generation (1, 11, 16, 75). Ravid *et al.* (50) demonstrated that Bcl-2 protects against H_2O_2- and C_6-ceramide-induced caspase-3 activation and cell death. In addition, Bcl-2 inhibited ceramide generation in response to inducers of apoptosis and also reduced the basal cellular levels of ceramide production, which suggests that the modulation of the ceramide pathway may be a target for the antiapoptotic effects of Bcl-2. Additional studies are required to determine precisely whether Bcl-2 prevents caspase activation directly or by inhibiting ceramide generation (Fig. 3). We hope that further investigations of the ceramide pathway, especially the identification of upstream and downstream components of ceramide signaling, will lead to a better understanding of the molecular mechanism by which Bcl-2 regulates apoptosis.

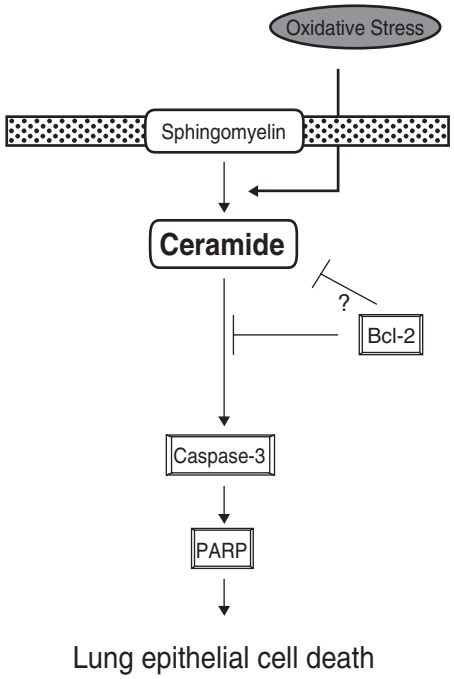

FIG. 3. Apoptotic events during ceramide-mediated apoptosis in A549 lung epithelial cells. Caspase-3 is activated following the induction of ceramide accumulation by oxidative stress, leading to PARP cleavage and apoptotic cell death. Bcl-2 inhibits the activation of caspase-3 and also prevents ceramide production.

Constitutive expression of high Bcl-2 protein levels has shown that Bcl-2 and related family members such as Bcl-x_L protect many cell types from apoptosis. Some death stimuli require BAX and BAK at the mitochondria, which may be an obligate control point for lipid second messengers and oxidative stimuli (18). However, the mechanisms of coordination between BAX and BAK are still unknown. It has been proposed that Bcl-2 may inhibit cell death by interfering with the function of proapoptotic Bcl-2 family members, by suppressing the release of cytochrome *c* from mitochondria into cytosol, by sequestering of caspase activators, such as Apaf-1, or by regulating calcium homeostasis (27). Therefore, several different explanations have been reported, but the exact biochemical functions carried out by Bcl-2 remain unknown (56).

The p53 tumor-suppressor gene initiates more than one pathway of apoptotic cell death (8, 14). It has been shown in Molt 4 leukemia cells that ceramide levels increase in response to irradiation in a p53-dependent manner (12). Moreover, both ceramide accumulation and GSH depletion induced by irradiation were dependent on p53 activation. However, induction of apoptosis can also be independent of activated p53. For example, Fleischer and Rebollo have recently reported that induction of apoptosis by integral membrane protein 2B, a BH3-only death protein of the Bcl-2 family, is p53-independent (19). Whether H_2O_2-induced ceramide generation in airway epithelial cells is p53-dependent is still unknown.

The role of ceramide in apoptosis has generated considerable debate (31, 32). Whereas caspases are widely described as the executioners of the program for cell death, the literature also conflicts with respect to the placement of ceramide relative to caspases in the apoptotic cascade (11, 61). However, Ravid *et al.* (50) showed that different stimuli acting at diverse sites to stimulate ceramide accumulation were able to trigger apoptosis, which supports the hypothesis that an increase in ceramide levels, *per se*, is sufficient to initiate the apoptotic cascade in lung epithelial cells. It was therefore concluded that ceramide elevation is a major cause for apoptosis induction and not just an outcome of cell death. Ceramide fits well upstream on the map of signaling mechanisms that lead to apoptosis in A549 airway epithelial cells.

RAFT CERAMIDE

Lipid rafts are liquid ordered state membrane domains rich in cholesterol and saturated polar lipids (usually sphingolipids). They can coexist with disordered fluid state membrane domains rich in unsaturated lipids (4, 52). Ceramide both stabilizes and associates strongly with lipid rafts (67, 72). It can also induce the formation of unusually large raft domains ("platforms") in plasma membranes (26, 45). Ceramide location within lipid rafts is an important factor in ceramide action (10, 25, 26, 28). It is possible that some ceramide effects are mediated by ceramide domains within the plasma membrane (34). In such a scenario, sphingomyelinase action and related hydrolysis of sphingomyelin to ceramide would change total membrane structure, apparently within caveolae (41) or other microdomains such as rafts and thereby induce signaling pathways (63).

Recent results indicate that ceramide-enriched membrane platforms serve to trap and cluster receptor molecules, for example, CD95 or CD40 (29). Ceramide-enriched membrane rafts may also, in some circumstances, serve to exclude receptor molecules and/or signaling proteins, facilitating concomitant activation of a specific signaling pathway and the inactivation of antagonistic signaling events.

It has recently been shown that gangliosides actively regulate growth factor receptor activity (46). Furthermore, it has been found that cholesterol depletion and subsequent release of the EGFR from rafts increased EGF binding to this receptor, indicating that raft association may have a negative regulatory role on EGFR activity (53). Therefore, a reorganization of signaling molecules with the exclusion of prosurvival molecules/receptors from ceramide-enriched membrane domains, and a recruitment of proapoptotic molecules into ceramide-enriched membrane domains might also mediate cell death and survival.

ROS MODULATE EGFR PHOSPHORYLATION, TRAFFICKING, AND CELL PROLIFERATION

Activation of EGFR by oxidants was shown to coincide with enhanced cell proliferation (65) and to facilitate tumorigenesis (35). Goldkorn *et al.* (23) have found that oxidants' targets at the plasma membrane are not only initiators of

apoptosis, such as nSMase (Fig. 1), but also membrane receptors, such as ErbB1, the EGFR that regulates the process of cell growth (2, 22, 24, 49). The molecular interactions between reactive oxidants and growth factor receptors, in particular EGFR, which play a key role in modulating cell proliferation signaling, may lead to aberrant growth control and airway epithelial hyperplasia (24, 64).

Inflammatory conditions are associated with increased production of ROS, as well as induction of NO synthesis. This is believed to play key roles in host defense but is also assumed to contribute to the development of tissue injury linked with chronic inflammation. $ONOO^-$, the reaction product of NO and O_2^-, has been suggested to significantly promote the pathophysiology of a large variety of diseases associated with inflammation (3). Van der Vliet *et al.* (64) have found that exposure to $ONOO^-$ generates a covalently cross-linked receptor most likely via intermolecular dityrosine cross-linking. $ONOO^-$ seems to preferentially cross-link activated EGFR, as shown by the increased degree of oxidant-induced covalent cross-linking after EGFR activation and by the elevated extent of tyrosine autophosphorylation in covalent EGFR dimers (64). Covalent EGFR dimerization may be of special importance in conditions of enhanced EGFR expression and activation, such as after epithelial injury or in malignant tumors, situations that are also frequently linked to elevated generation of NO and/or inflammatory oxidants. Irreversible EGFR dimerization may possibly alter receptor activation and downstream signaling and could thereby affect epithelial repair processes or tumor development.

Whereas $ONOO^-$ directly activates EGFR by covalently cross-linking the receptor (64), H_2O_2 phosphorylates EGFR only on tyrosines, thereby creating an aberrantly phosphorylated receptor with a longer half-life (24). Unlike EGF and $ONOO^-$, H_2O_2 failed to induce detectable dimerization of EGFR. We found that H_2O_2 induced EGFR phosphorylation in a manner dependent both on c-Src and on EGFR kinase activation, but not on EGFR dimerization. Furthermore, EGFR activation by H_2O_2 was ligand-independent, as blocking the EGFR ligand binding site with anti-EGFR monoclonal antibody 225 did not interfere with H_2O_2-mediated receptor activation (Goldkorn *et al.*, unpublished observations). It should be noted that ROS also cause the proliferation of lung epithelial and mesenchymal cells that does not result in malignant transformation and occurs via the release of soluble EGFR ligands. For example, H_2O_2 may stimulate the production of EGFR ligands, including the heparin-bound-EGF-like ligand to EGFR (38). Therefore, H_2O_2 may stimulate EGFR activation through both ligand-dependent and ligand-independent mechanisms.

Activation of the EGFR by its ligands is followed by desensitization processes, which involve removal of the activated receptors from the cell surface ("down-regulation") (68). Interaction of the receptor with the ubiquitin ligase, c-Cbl, has been shown to be critical for effective receptor down-regulation (43). Recently, this interaction has also been shown to depend on receptor phosphorylation at tyrosine 1045, the major docking site for c-Cbl (44). Following interaction, c-Cbl is phosphorylated, resulting in the activation of its ubiquitin ligase activity. This promotes the recruitment of the ubiquitin-conjugating enzyme UbcH7 (73) and consequent receptor ubiquitylation, which targets the receptor for proteasomal/lysosomal degradation (40, 43, 44, 73). Recent studies (13, 59) have shown that c-Cbl also regulates EGFR endocytosis by entailing the creation of a multiprotein "endocytotic complex" with activated EGFR at the plasma membrane, thus controlling receptor internalization. Hence, the recruitment of c-Cbl to the EGFR appears to be an essential step in receptor down-regulation. By mediating receptor endocytosis and "tagging" it with ubiquitin molecules, c-Cbl targets the receptor for lysosomal and/or proteasomal degradation (Fig. 4).

As the oncogenic action of the EGFR correlates with its overexpression at the plasma membrane (15, 20), removal of activated receptor molecules from the cell surface by sorting for degradation is expected to inhibit their oncogenic potential. Indeed, Goldkorn *et al.* (24) found that exposures to oxidative stress in the form of H_2O_2 preferentially enhanced tyrosine phosphorylation of the EGFR, creating an activated but not down-regulated receptor. Furthermore, the results presented by Ravid *et al.* (49) indicate that although the EGFR is tyrosine-phosphorylated under H_2O_2 oxidative stress, phosphorylation at tyrosine residue 1045 is abrogated, and therefore it fails to initiate c-Cbl-mediated receptor degradation.

Additional support for this model is provided in recent studies by Yarden and co-workers (44, 69), who demonstrated that a mutant EGFR, whose tyrosine 1045 was changed to phenylalanine (Y1045F), lost its ability to undergo ubiquitylation and degradation upon induction with EGF. In an analogy to the tyrosine 1045 mutant, Ravid *et al.* (49) have shown that activation of EGFR by oxidative stress fails to phosphorylate tyrosine 1045, while enhancing the phosphorylation of other tyrosine residues, such as tyrosines 845 and 1173. This resulted in a different intracellular distribution of the receptor. Whereas following EGF treatment of A549 lung epithelial cells the receptor displayed a pattern of vesicles throughout the cytoplasm, under H_2O_2 exposure the EGFR stayed mainly at the plasma membrane (Fig. 5). This contributes to prolonged downstream signaling, as demonstrated by persistent phosphorylation of extracellular signal-regulated kinase (ERK) 1/2 (Fig. 6). Therefore, H_2O_2 exposure may cause a pattern of tyrosine phosphorylation similar to that of the mutant receptor (Y1045F). In both cases, tyrosine 1045 is not phosphorylated, and the receptor is defective in ubiquitylation and degradation. Thus, upon H_2O_2 activation, the receptor becomes aberrantly phosphorylated and is incapable of recruiting c-Cbl. This in turn confers prolonged signaling of the receptor (Fig. 7).

These findings fit well with the paradigm suggesting that the oncogenic action of the EGFR depends on its accumulation at the plasma membrane. The failure of activated EGFR to be degraded in a ubiquitin-mediated fashion is an unexpected response to H_2O_2, contradicting the general notion that oxidative stress results in rapid removal of oxidized proteins by the ubiquitin-proteasome system (9, 17, 58).

CONCLUSIONS

As ROS have diverse activities, they can function as cell toxins or signaling molecules. In the latter role, they may affect various cell functions ranging from cellular proliferation

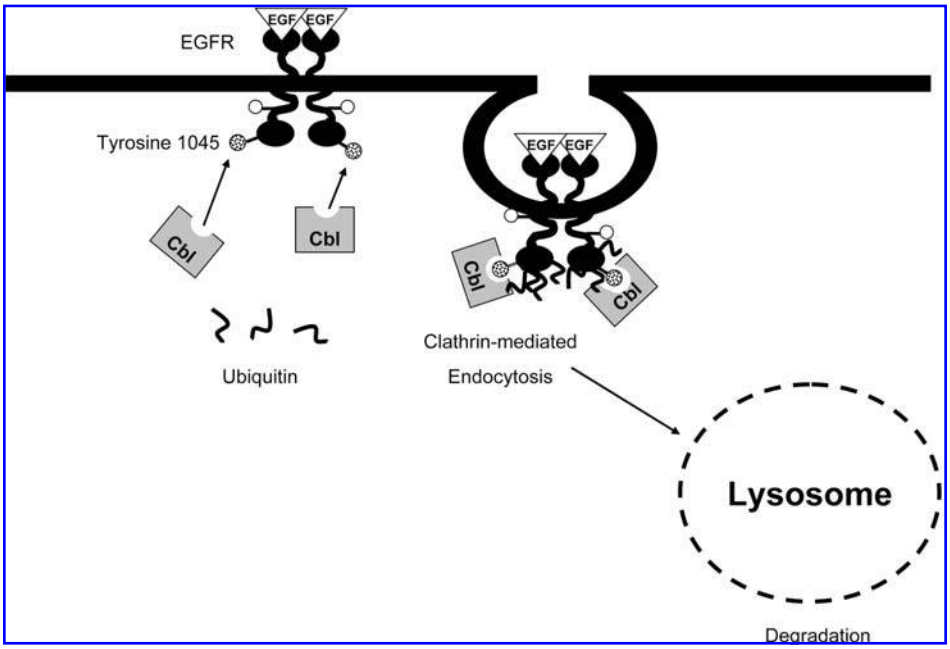

FIG. 4. c-Cbl targets the EGFR for lysosomal and/or proteasomal degradation.

to apoptosis. What determines which of the numerous activities of ROS will prevail is still unknown. The extent of ROS production, the site and source of their generation, the cell type, and the antioxidant status of the cell all likely affect the final outcome.

Although it is unequivocal that oxidative stress modulates ceramide production, many of the links between oxidative stress and ceramide signaling are still correlative and require rigorous molecular and mechanistic studies. The exact molecular mechanisms and the subcellular localization of oxidative stress and ceramide pathway(s) interactions need to be defined. The molecular identification of sphingomyelin that are modulated by oxidative stress is not complete. Recent studies reported the cloning of two candidate nSMases (33), but evidence was provided that one nSMase localizes to the endoplasmic reticulum and functions as a lyso-platelet-activating factor phospholipase C (55), whereas the other nSMase localizes to the Golgi and its physiologic substrates were not established. Because enzymatic and subfractionation studies indicate the presence of a plasma membrane nSMase that is modulated by oxidative stress, effort is still being directed toward isolating and characterizing this sphingomyelinase from the lung (Goldkorn *et al.*, unpublished observations). Furthermore, most of the key enzymes regulating ceramide metabolism have not yet been characterized. Therefore, there is still a lack of molecular and pharmacological tools to study these pathways and their functions.

On the one hand, it is desired to prevent the killing of cells and tissue injury that oxidant–ceramide coupling leads to in diseases such as asthma or ARDS. On the other hand, in cancer, the opposite effect is sought. But the proliferation of cancer cells depends not only on locally produced growth fac-

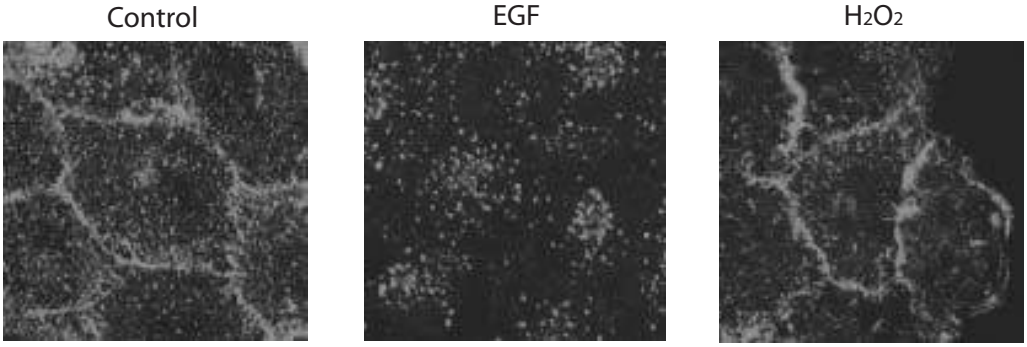

FIG. 5. Intracellular distribution of the EGFR during exposure to oxidative stress. Serum-starved A549 cells, grown on coverslips, were incubated with or without 200 μ*M* H_2O_2 or 100 ng/ml EGF for 15 min. After fixation, cells were incubated with anti-EGFR antibody 528 followed by Alexa Fluor 488-conjugated secondary antibody staining. Analysis was performed by confocal microscopy.

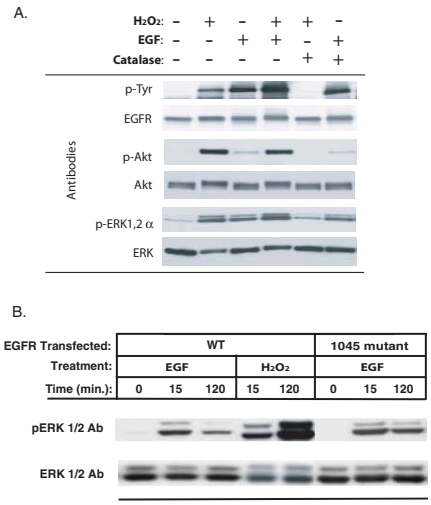

FIG. 6. Hyperplastic responses to H_2O_2. (**A**) Downstream activation of ERKs and Akt phosphorylation. Cells were exposed to the various treatments for 15 min with 1 U/ml glucose oxidase (to generate H_2O_2) or 100 ng/ml EGF in the presence or absence of 2,500 U/ml catalase. Cell lysates were subjected to SDS-PAGE and immunoblotted with the indicated antibodies. (**B**) A mutant EGFR defective at tyrosine 1,045 elicits potent hyperplastic and oncogenic signals: persistence of ERK signaling. CHO cells were transiently transfected with either wild-type EGFR (WT) or a tyrosine 1045 mutant (Y1045F). After 24 h of starvation, the cells were treated with either 100 ng/ml EGF or 1 U/ml glucose oxidase for the indicated time points. Cell lysates were subjected to SDS-PAGE and immunoblotting with anti-ERK and anti-p-ERK antibodies. The cell lysis buffer used is described in Fig. 1B. Anti-phosphotyrosine (PY20) antibody was from Santa Cruz Biotechnology (Santa Cruz, CA, U.S.A.), anti-EGFR (RK2) antibody was a generous gift from Dr. J. Schlessinger (New York University Medical Center, NY, NY, U.S.A.), and the remaining antibodies were from Cell Signaling Technology (Beverly, MA).

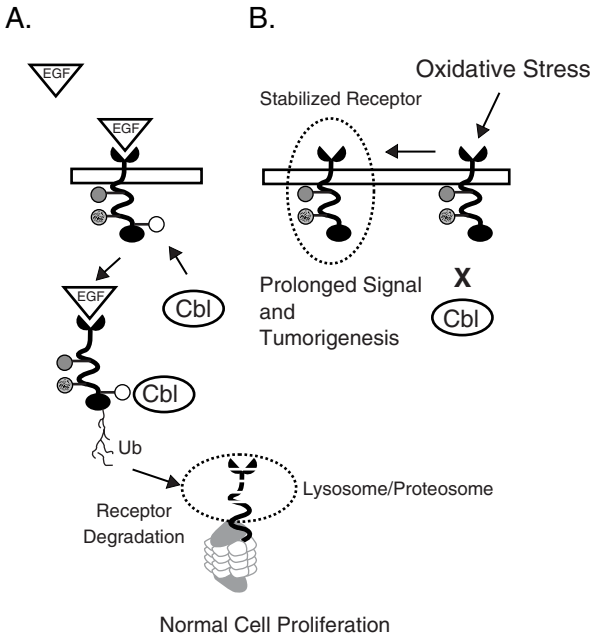

FIG. 7. Activation of EGFR during exposures to ligand (EGF) or to oxidative stress (H_2O_2). (**A**) When exposed to EGF, the receptor is phosphorylated, and c-Cbl is recruited, leading to receptor down-regulation and normal cell proliferation. (**B**) When exposed to H_2O_2, the receptor is aberrantly phosphorylated, tyrosine 1,045 is not phosphorylated, c-Cbl is not recruited, and the receptor is not down-regulated, leading to prolonged signaling that may cause tumorigenesis.

tors. Oxidants may also activate the growth factor receptors, enhancing cell proliferation and facilitating tumor promotion processes (35, 65).

Signaling by growth factors involves phosphorylation events, and its termination is determined mainly by endocytosis of growth factor receptor complexes. However, our recent studies suggest that during oxidative stress the majority of activated EGFR reside at the plasma membrane. The differences in localization may have implications in both the magnitude of the EGFR response and its outcome (70). Furthermore, it has been shown that preventing EGFR down-regulation facilitates cell proliferation and transformation (43, 66, 69). Therefore, our data showing that exposure to H_2O_2 elicits delayed EGFR activation with prolonged duration at the plasma membrane offer additional support to the oncogenic ability of H_2O_2, which had been demonstrated to be EGFR-dependent (35). This accumulation of activated EGFR during oxidative stress may provide the trigger for cell hyperplasia. The emerging role of H_2O_2 in EGFR signaling may also be relevant to other receptor tyrosine kinases that undergo c-Cbl-mediated down-regulation, and this is currently under investigation.

Moreover, ROS and ceramide generation may enable the uptake of certain receptors (*e.g.*, growth factor receptors) into special raft areas, whereas other receptors may be excluded. Additionally, a reorganization of signaling molecules wtihin ceramide-enriched membrane domains, wherein prosurvival molecules are excluded and proapoptotic molecules are recruited, might also regulate the survival and death of the cells. In summary, elucidating the exact molecular interactions between oxidative stress and ceramide pathways, raft formation, and the detailed understanding of ubiquitylation control on receptor desensitization under oxidative stress should lead to new strategies for pharmacological intervention in diverse pulmonary diseases and provide better ways to diagnose and eradicate cancer.

ABBREVIATIONS

ARDS, acute respiratory distress syndrome; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; ELF, epithelial lining fluid; ERK, extracullular signal-regulated kinase; GSH, reduced glutathione; GSSG, oxidized glutathione; H_2O_2, hydrogen peroxide; NO, nitric oxide; nSMase, neutral sphingomyelinase; O_2^-, superoxide anion; $ONOO^-$, peroxynitrite; PARP, poly(ADP-ribose) polymerase; ROS, reactive oxygen species; SDS-PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; TBE, tracheobronchial epithelial cells.

REFERENCES

1. Allouche M, Bettaieb A, Vindis C, Rousse A, Grignon C, and Laurent G. Influence of Bcl-2 overexpression on the ceramide pathway in daunorubicin-induced apoptosis of leukemic cells. *Oncogene* 14: 1837–1845, 1997.
2. Balaban N, Moni J, Shannon M, Dang L, Murphy E, and Goldkorn T. The effect of ionizing radiation on signal transduction: antibodies to EGF receptor sensitize A431 cells to radiation. *Biochim Biophys Acta* 1314: 147–156, 1996.
3. Beckman JS and Koppenol WH. Nitric oxide, superoxide, and peroxynitrite: the good, the bad, and ugly. *Am J Physiol* 271: C1424–C1437, 1996.
4. Brown DA and London E. Structure and origin of ordered lipid domains in biological membranes. *J Membr Biol* 164: 103–114, 1998.
5. Brune B, Sandau K, and von Knethen A. Apoptotic cell death and nitric oxide: activating and antagonistic transducing pathways. *Biochemistry* (Mosc) 63: 817–825, 1998.
6. Brune B, von Knethen A, and Sandau KB. Nitric oxide and its role in apoptosis. *Eur J Pharmacol* 351: 261–272, 1998.
7. Chan C and Goldkorn T. Ceramide path in human lung cell death. *Am J Respir Cell Mol Biol* 22: 460–468, 2000.
8. Chao C, Saito S, Kang J, Anderson CW, Appella E, and Xu Y. p53 transcriptional activity is essential for p53-dependent apoptosis following DNA damage. *EMBO J* 19: 4967–4975, 2000.
9. Cooper KF, Mallory MJ, and Strich R. Oxidative stress-induced destruction of the yeast C-type cyclin Ume3p requires phosphatidylinositol-specific phospholipase C and the 26S proteasome. *Mol Cell Biol* 19: 3338–3348, 1999.
10. Cremesti AE, Goni FM, and Kolesnick R. Role of sphingomyelinase and ceramide in modulating rafts: do biophysical properties determine biologic outcome? *FEBS Lett* 531: 47–53, 2002.
11. Dbaibo GS, Perry DK, Gamard CJ, Platt R, Poirier GG, Obeid LM, and Hannun YA. Cytokine response modifier A (CrmA) inhibits ceramide formation in response to tumor necrosis factor (TNF)-alpha: CrmA and Bcl-2 target distinct components in the apoptotic pathway. *J Exp Med* 185: 481–490, 1997.
12. Dbaibo GS, Pushkareva MY, Rachid RA, Alter N, Smyth MJ, Obeid LM, and Hannun YA. p53-dependent ceramide response to genotoxic stress. *J Clin Invest* 102: 329–339, 1998.
13. de Melker AA, van der Horst G, Calafat J, Jansen H, and Borst J. c-Cbl ubiquitinates the EGF receptor at the plasma membrane and remains receptor associated throughout the endocytic route. *J Cell Sci* 114: 2167–2178, 2001.
14. Do TN, Rosal RV, Drew L, Raffo AJ, Michl J, Pincus MR, Friedman FK, Petrylak DP, Cassai N, Szmulewicz J, Sidhu G, Fine RL, and Brandt-Rauf PW. Preferential induction of necrosis in human breast cancer cells by a p53 peptide derived from the MDM2 binding site. *Oncogene* 22: 1431–1444, 2003.
15. Eccles SA, Modjtahedi H, Box G, Court W, Sandle J, and Dean CJ. Significance of the c-erbB family of receptor tyrosine kinases in metastatic cancer and their potential as targets for immunotherapy. *Invasion Metastasis* 14: 337–348, 1994.
16. El-Assaad W, El-Sabban M, Awaraji C, Abboushi N, and Dbaibo GS. Distinct sites of action of Bcl-2 and Bcl-xL in the ceramide pathway of apoptosis. *Biochem J* 336: 735–741, 1998.
17. Figueiredo-Pereira ME and Cohen G. The ubiquitin/proteasome pathway: friend or foe in zinc-, cadmium-, and H_2O_2-induced neuronal oxidative stress. *Mol Biol Rep* 26: 65–69, 1999.
18. Finkel T and Holbrook NJ. Oxidants, oxidative stress and the biology of ageing. *Nature* 408: 239–247, 2000.
19. Fleischer A and Rebollo A. Induction of p53-independent apoptosis by the BH3-only protein ITM2Bs. *FEBS Lett* 557: 283–287, 2004.
20. Franklin WA, Veve R, Hirsch FR, Helfrich BA, and Bunn PA Jr. Epidermal growth factor receptor family in lung cancer and premalignancy. *Semin Oncol* 29: 3–14, 2002.
21. Garcia-Ruiz C, Colell A, Mari M, Morales A, and Fernandez-Checa JC. Direct effect of ceramide on the mitochondrial electron transport chain leads to generation of reactive oxygen species. Role of mitochondrial glutathione. *J Biol Chem* 272: 11369–11377, 1997.
22. Goldkorn T, Balaban N, Shannon M, and Matsukuma K EGF receptor phosphorylation is affected by ionizing radiation. *Biochim Biophys Acta* 1358: 289–299, 1997.
23. Goldkorn T, Balaban N, Shannon M, Chea V, Matsukuma K, Gilchrist D, Wang H, and Chan C. H_2O_2 acts on cellular membranes to generate ceramide signaling and initiate apoptosis in tracheobronchial epithelial cells. *J Cell Sci* 111: 3209–3220, 1998.
24. Goldkorn T, Balaban N, Matsukuma K, Chea V, Gould R, Last J, Chan C, and Chavez C. EGF-receptor phosphorylation and signaling are targeted by H_2O_2 redox stress. *Am J Respir Cell Mol Biol* 19: 786–798, 1998.
25. Grassme H, Jekle A, Riehle A, Schwarz H, Berger J, Sandhoff K, Kolesnick R, and Gulbins E. CD95 signaling via ceramide-rich membrane rafts. *J Biol Chem* 276: 20589–20596, 2001.
26. Grassme H, Jendrossek V, Riehle A, von Kurthy G, Berger J, Schwarz H, Weller M, Kolesnick R, and Gulbins E. Host defense against *Pseudomonas aeruginosa* requires ceramide-rich membrane rafts. *Nat Med* 9: 322–330, 2003.
27. Green DR and Reed JC. Mitochondria and apoptosis. *Science* 281: 1309–1312, 1998.
28. Gulbins E. Regulation of death receptor signaling and apoptosis by ceramide. *Pharmacol Res* 47: 393–399, 2003.
29. Gulbins E and Kolesnick R. Raft ceramide in molecular medicine. *Oncogene* 22: 7070–7077, 2003.
30. Halliwell B and Gutteridge JM. Role of free radicals and catalytic metal ions in human disease: an overview. *Methods Enzymol* 186: 1–85, 1990.
31. Hannun YA and Luberto C. Ceramide in the eukaryotic stress response. *Trends Cell Biol* 10: 73–80, 2000.
32. Hofmann K and Dixit VM. Ceramide in apoptosis—does it really matter? *Trends Biochem Sci* 23: 374–377, 1998.
33. Hofmann K, Tomiuk S, Wolff G, and Stoffel W. Cloning and characterization of the mammalian brain-specific, Mg^{2+}-dependent neutral sphingomyelinase. *Proc Natl Acad Sci U S A* 97: 5895–5900, 2000.

34. Holopainen JM, Subramanian M, and Kinnunen PK. Sphingomyelinase induces lipid microdomain formation in a fluid phosphatidylcholine/sphingomyelin membrane. *Biochemistry* 37: 17562–17570, 1998.
35. Huang RP, Peng A, Golard A, Hossain MZ, Huang R, Liu YG, and Boynton AL. Hydrogen peroxide promotes transformation of rat liver non-neoplastic epithelial cells through activation of epidermal growth factor receptor. *Mol Carcinog* 30: 209–217, 2001.
36. Huwiler A, Pfeilschifter J, and van den Bosch H. Nitric oxide donors induce stress signaling via ceramide formation in rat renal mesangial cells. *J Biol Chem* 274: 7190–7195, 1999.
37. Huwiler A, Kolter T, Pfeilschifter J, and Sandhoff K. Physiology and pathophysiology of sphingolipid metabolism and signaling. *Biochim Biophys Acta* 1485: 63–99, 2000.
38. Ingram JL, Rice AB, Santos J, Van Houten B, and Bonner JC. Vanadium-induced HB-EGF expression in human lung fibroblasts is oxidant dependent and requires MAP kinases. *Am J Physiol Lung Cell Mol Physiol* 284: L774–L782, 2003.
39. Jaffrezou JP, Maestre N, de Mas-Mansat V, Bezombes C, Levade T, and Laurent G. Positive feedback control of neutral sphingomyelinase activity by ceramide. *FASEB J* 12: 999–1006, 1998.
40. Joazeiro CA, Wing SS, Huang H, Leverson JD, Hunter T, and Liu YC. The tyrosine kinase negative regulator c-Cbl as a RING-type, E2-dependent ubiquitin-protein ligase. *Science* 286: 309–312, 1999.
41. Kurzchalia TV and Parton RG. Membrane microdomains and caveolae. *Curr Opin Cell Biol* 11: 424–431, 1999.
42. Lavrentiadou SN, Chan C, Kawcak TN, Ravid T, Tsaba A, van der Vliet A, Rasooly R, and Goldkorn T. Ceramide-mediated apoptosis in lung epithelial cells is regulated by glutathione. *Am J Respir Cell Mol Biol* 25: 676–684, 2001.
43. Levkowitz G, Waterman H, Zamir E, Kam Z, Oved S, Langdon WY, Beguinot L, Geiger B, and Yarden Y. c-Cbl/Sli-1 regulates endocytic sorting and ubiquitination of the epidermal growth factor receptor. *Genes Dev* 12: 3663–3674, 1998.
44. Levkowitz G, Waterman H, Ettenberg SA, Katz M, Tsygankov AY, Alroy I, Lavi S, Iwai K, Reiss Y, Ciechanover A, Lipkowitz S, and Yarden Y. Ubiquitin ligase activity and tyrosine phosphorylation underlie suppression of growth factor signaling by c-Cbl/Sli-1. *Mol Cell* 4: 1029–1040, 1999.
45. London M and London E. Ceramide selectively displaces cholesterol from ordered lipid domains (Rafts): implications for lipid raft structure and function. *J Biol Chem* 279: 9997–10004, 2003.
46. Miljan EA and Bremer EG. Regulation of growth factor receptors by gangliosides. *Sci STKE* 2002: RE15, 2002.
47. Nowak D, Antczak A, Krol M, Pietras T, Shariati B, Bialasiewicz P, Jeczkowski K, and Kula P. Increased content of hydrogen peroxide in the expired breath of cigarette smokers. *Eur Respir J* 9: 652–657, 1996.
48. Pautz A, Franzen R, Dorsch S, Boddinghaus B, Briner VA, Pfeilschifter J, and Huwiler A. Cross-talk between nitric oxide and superoxide determines ceramide formation and apoptosis in glomerular cells. *Kidney Int* 61: 790–796, 2002.
49. Ravid T, Sweeney C, Gee P, Carraway KL III, and Goldkorn T. Epidermal growth factor receptor activation under oxidative stress fails to promote c-Cbl mediated down-regulation. *J Biol Chem* 277: 31214–31219, 2002.
50. Ravid T, Tsaba A, Gee P, Rasooly R, Medina EA, and Goldkorn T. Ceramide accumulation precedes caspase-3 activation during apoptosis of A549 human lung adenocarcinoma cells. *Am J Physiol Lung Cell Mol Physiol* 284: L1082–L1092, 2003.
51. Reed JC. Bcl-2 and the regulation of programmed cell death. *J Cell Biol* 124: 1–6, 1994.
52. Rietveld A and Simons K. The differential miscibility of lipids as the basis for the formation of functional membrane rafts. *Biochim Biophys Acta* 1376: 467–479, 1998.
53. Roepstorff K, Thomsen P, Sandvig K, and van Deurs B. Sequestration of epidermal growth factor receptors in non-caveolar lipid rafts inhibits ligand binding. *J Biol Chem* 277: 18954–18960, 2002.
54. Sawada M, Nakashima S, Banno Y, Yamakawa H, Takenaka K, Shinoda J, Nishimura Y, Sakai N, and Nozawa Y. Influence of Bax or Bcl-2 overexpression on the ceramide-dependent apoptotic pathway in glioma cells. *Oncogene* 19: 3508–3520, 2000.
55. Sawai H, Domae N, Nagan N, and Hannun YA. Function of the cloned putative neutral sphingomyelinase as lyso-platelet activating factor-phospholipase C. *J Biol Chem* 274: 38131–38139, 1999.
56. Scorrano L, Oakes SA, Opferman JT, Cheng EH, Sorcinelli MD, Pozzan T, and Korsmeyer SJ. BAX and BAK regulation of endoplasmic reticulum Ca^{2+}: a control point for apoptosis. *Science* 300: 135–139, 2003.
57. Sen CK and Packer L. Antioxidant and redox regulation of gene transcription. *FASEB J* 10: 709–720, 1996.
58. Shang F, Gong X, and Taylor A. Activity of ubiquitin-dependent pathway in response to oxidative stress. Ubiquitin-activating enzyme is transiently up-regulated. *J Biol Chem* 272: 23086–23093, 1997.
59. Soubeyran P, Kowanetz K, Szymkiewicz I, Langdon WY, and Dikic I. Cbl-CIN85-endophilin complex mediates ligand-induced downregulation of EGF receptors. *Nature* 416: 183–187, 2002.
60. Sznajder JI, Fraiman A, Hall JB, Sanders W, Schmidt G, Crawford G, Nahum A, Factor P, and Wood LD. Increased hydrogen peroxide in the expired breath of patients with acute hypoxemic respiratory failure. *Chest* 96: 606–612, 1989.
61. Takeda Y, Tashima M, Takahashi A, Uchiyama T, and Okazaki T. Ceramide generation in nitric oxide-induced apoptosis. Activation of magnesium-dependent neutral sphingomyelinase via caspase-3. *J Biol Chem* 274: 10654–10660, 1999.
62. Tepper AD, de Vries E, van Blitterswijk WJ, and Borst J. Ordering of ceramide formation, caspase activation, and mitochondrial changes during CD95- and DNA damage-induced apoptosis [published erratum appears in *J Clin Invest* 103:1363, 1999]. *J Clin Invest* 103: 971–978, 1999.
63. van Blitterswijk WJ. Hypothesis: ceramide conditionally activates atypical protein kinases C, Raf-1 and KSR through binding to their cysteine-rich domains. *Biochem J* 331 (Pt 2): 679–680, 1998.

64. van der Vliet A, Hristova M, Cross CE, Eiserich JP, and Goldkorn T. Peroxynitrite induces covalent dimerization of epidermal growth factor receptors in A431 epidermoid carcinoma cells. *J Biol Chem* 273: 31860–31866, 1998.
65. Van Winkle LS, Isaac JM, and Plopper CG. Distribution of epidermal growth factor receptor and ligands during bronchiolar epithelial repair from naphthalene-induced Clara cell injury in the mouse. *Am J Pathol* 151: 443–459, 1997.
66. Vieira AV, Lamaze C, and Schmid SL. Control of EGF receptor signaling by clathrin-mediated endocytosis. *Science* 274: 2086–2089, 1996.
67. Wang TY and Silvius JR. Sphingolipid partitioning into ordered domains in cholesterol-free and cholesterol-containing lipid bilayers. *Biophys J* 84: 367–378, 2003.
68. Waterman H and Yarden Y. Molecular mechanisms underlying endocytosis and sorting of ErbB receptor tyrosine kinases. *FEBS Lett* 490: 142–152, 2001.
69. Waterman H, Katz M, Rubin C, Shtiegman K, Lavi S, Elson A, Jovin T, and Yarden Y. A mutant EGF-receptor defective in ubiquitylation and endocytosis unveils a role for Grb2 in negative signaling. *EMBO J* 21: 303–313, 2002.
70. Wiley HS and Burke PM. Regulation of receptor tyrosine kinase signaling by endocytic trafficking. *Traffic* 2: 12–18, 2001.
71. Williams MD and Chance B. Spontaneous chemiluminescence of human breath. Spectrum, lifetime, temporal distribution, and correlation with peroxide. *J Biol Chem* 258: 3628–3631, 1983.
72. Xu X, Bittman R, Duportail G, Heissler D, Vilcheze C, and 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* 276: 33540–33546, 2001.
73. Yokouchi M, Kondo T, Houghton A, Bartkiewicz M, Horne WC, Zhang H, Yoshimura A, and Baron R. Ligand-induced ubiquitination of the epidermal growth factor receptor involves the interaction of the c-Cbl RING finger and UbcH7. *J Biol Chem* 274: 31707–31712, 1999.
74. Yoshimura S, Banno Y, Nakashima S, Takenaka K, Sakai H, Nishimura Y, Sakai N, Shimizu S, Eguchi Y, Tsujimoto Y, and Nozawa Y. Ceramide formation leads to caspase-3 activation during hypoxic PC12 cell death. Inhibitory effects of Bcl-2 on ceramide formation and caspase-3 activation. *J Biol Chem* 273: 6921–6927, 1998.
75. Zhang J, Alter N, Reed JC, Borner C, Obeid LM, and Hannun YA. Bcl-2 interrupts the ceramide-mediated pathway of cell death. *Proc Natl Acad Sci U S A* 93: 5325–5328, 1996.

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2. J. Yang, Q. Li, X. D. Zhou, V. P. Kolosov, J. M. Perelman. 2011. Naringenin attenuates mucous hypersecretion by modulating reactive oxygen species production and inhibiting NF-#B activity via EGFR-PI3K-Akt/ERK MAPKinase signaling in human airway epithelial cells. *Molecular and Cellular Biochemistry* **351**:1-2, 29-40. [CrossRef]
3. Simone Reuter, Subash C. Gupta, Madan M. Chaturvedi, Bharat B. Aggarwal. 2010. Oxidative stress, inflammation, and cancer: How are they linked?. *Free Radical Biology and Medicine* **49**:11, 1603-1616. [CrossRef]
4. Subhadip Raychaudhuri, Eric Willgohs, Thuc-Nghi Nguyen, Elaine M. Khan, Tzipora Goldkorn. 2008. Monte Carlo Simulation of Cell Death Signaling Predicts Large Cell-to-Cell Stochastic Fluctuations through the Type 2 Pathway of Apoptosis. *Biophysical Journal* **95**:8, 3559-3562. [CrossRef]
5. Henry Jay Forman, Jon M. Fukuto, Tom Miller, Hongqiao Zhang, Alessandra Rinna, Smadar Levy. 2008. The chemistry of cell signaling by reactive oxygen and nitrogen species and 4-hydroxynonenal. *Archives of Biochemistry and Biophysics* **477**:2, 183-195. [CrossRef]
6. Jianguo Lin, Anping Chen. 2008. Activation of peroxisome proliferator-activated receptor-# by curcumin blocks the signaling pathways for PDGF and EGF in hepatic stellate cells. *Laboratory Investigation* **88**:5, 529-540. [CrossRef]
7. Norman J Haughey. 2007. Inflammatory, oxidative and lipid perspectives on dementia in HIV-infected patients. *Biomarkers in Medicine* **1**:2, 221-224. [CrossRef]
8. Mai A Ngo, Nadezda N Sinitsyna, Qin Qin, Robert H Rice. 2007. Oxygen-Dependent Differentiation of Human Keratinocytes. *Journal of Investigative Dermatology* **127**:2, 354-361. [CrossRef]
9. Gisela Guti??rrez, Criselda Mendoza, Luis F. Monta??o, Rebeca L??pez-Marure. 2007. Ceramide induces early and late apoptosis in human papilloma virus+ cervical cancer cells by inhibiting reactive oxygen species decay, diminishing the intracellular concentration of glutathione and increasing nuclear factor-??B translocation. *Anti-Cancer Drugs* **18**:2, 149-159. [CrossRef]
10. L YU, S VENKATARAMAN, M COLEMAN, D SPITZ, P WERTZ, F DOMANN. 2006. Glutathione peroxidase-1 inhibits UVA-induced AP-2# expression in human keratinocytes. *Biochemical and Biophysical Research Communications* **351**:4, 1066-1071. [CrossRef]
11. M LEVY, S CASTILLO, T GOLDKORN. 2006. nSMase2 activation and trafficking are modulated by oxidative stress to induce apoptosis#. *Biochemical and Biophysical Research Communications* **344**:3, 900-905. [CrossRef]
12. Dr. Irfan Rahman , Se-Ran Yang , Saibal K. Biswas . 2006. Current Concepts of Redox Signaling in the Lungs. *Antioxidants & Redox Signaling* **8**:3-4, 681-689. [Abstract] [Full Text PDF] [Full Text PDF with Links]
13. Jae-Myung Yoo, Youn-Sun Lee, Heon-Kyo Choi, Yong-Moon Lee, Jin-Tae Hong, Yeo-Pyo Yun, Seikwan Oh, Hwan-Soo Yoo. 2005. Protection of LLC-PK1 cells against hydrogen peroxide-induced cell death by modulation of ceramide level. *Archives of Pharmacal Research* **28**:3, 311-318. [CrossRef]
14. Irfan Rahman . 2005. Redox Signaling in the Lungs. *Antioxidants & Redox Signaling* **7**:1-2, 1-5. [Citation] [Full Text PDF] [Full Text PDF with Links]