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Forum Review

Anchorage-Dependent Cell Growth: Tyrosine Kinases and Phosphatases Meet Redox Regulation

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ABSTRACT

Recent data have provided new insight concerning the regulation of nontransformed cell proliferation in response to both soluble growth factors and adhesive cues. Nontransformed cells are anchorage-dependent for the execution of the complete mitotic program and cannot avoid the concomitant signals starting from mitogenic molecules, as growth factors, and adhesive agents belonging to the extracellular matrix. Protein tyrosine kinases (PTKs) and phosphotyrosine phosphatases (PTPs) together with soluble small molecules have been included among intracellular signal transducers of growth factor and extracellular matrix receptors. Reactive oxygen species retain a key role during both growth factor and integrin receptor signaling, and these second messengers are recognized to be a synergistic point of confluence for anchorage-dependent growth signaling. Redox-regulated proteins include PTPs and PTKs, although with opposite regulation of enzymatic activity. Transient oxidation of PTPs leads to their inactivation, through the formation of an intramolecular S-S bridge. Conversely, oxidation of PTKs leads to their activation, either by direct SH modification or, indirectly, by concomitant inhibition of PTPs that leads to sustained activation of PTKs. This review will focus on the redox regulation of PTPs and PTKs during anchorage-dependent cell growth and its implications for tumor biology. *Antioxid. Redox Signal.* 7, 578–592.

ANCHORAGE-DEPENDENT GROWTH CONTROL

SIGNALS FROM DYNAMIC CELLULAR INTERACTIONS between the extracellular matrix (ECM) and neighboring cells ultimately input into the cellular decision-making development. These interactions form the starting point of anchorage-dependent growth. Current advances have provided the mechanistic details behind the ability of cell adhesion molecules, among them integrins, to regulate both early signal transduction events initiated by soluble factors and downstream events more proximally involved in cell-cycle progression. These actions appear to depend on the ability of adhesion molecules to initiate the formation of organized structures that permit the efficient flow of information (25).

Cell proliferation is controlled not only by soluble growth factors (GFs), but also by components of the ECM, such as fibronectin and laminin, to which cells adhere via the integrin family of receptors. Input from both GF receptors and integrins is required to stimulate progression through the G1 phase of the cell cycle, via induction of G1 cyclins and suppression of inhibitors of the G1 cyclin-dependent kinases (39). Extensive cross talk takes place between integrin and GF receptor signaling pathways, and mitogenic signaling is weak and transient in the absence of integrin-mediated cell adhesion. In normal untransformed cells, all of the important mitogenic signal transduction cascades, namely those downstream of the Ras and Rho family small GTPases and the phosphatidylinositol 3-kinase (PI3K)/Akt pathway, are regulated by integrin-mediated cell adhesion (22). As a result, these cells are anchorage-dependent for growth. In contrast, constitutive activity of each of these routes has been described in cancer cells, which not only reduces their GF dependence, but also allows these cells to grow in an anchorage-independent fashion. Hence, the ability to proliferate in

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the absence of anchorage is a fundamental feature of cancer cells, yet how it is acquired is one central problem in cancer biology.

The integrin receptors and their signaling

Integrins are heterodimers of α and β subunits. Eight β and 18 α subunits may combine to form 24 distinct integrins. Although each integrin has its own binding specificity, many of them bind to the same ligand or to partially overlapping sets of ligands, leading to multiple integrin recognition of the major adhesive components of the ECM, namely fibronectin and laminin (40).

Integrins transmit signals that inhibit apoptosis, regulate progression through the G1 phase of the cell cycle, and promote either stable adhesion or migration (84). The key features of integrin signaling are (Fig. 1): (a) their association with elements of the cytoskeleton, leading many of their signaling functions to be devoted to its organization; (b) the persistence of their functional effects over time, due to the non-diffusibility of most matrix ligands and to integrin long half-life on the plasma membrane; and (c) their signaling through adaptor proteins, namely protein and lipid kinases and small GTPases, due to their short cytoplasmic domains and lack of intrinsic catalytic activity.

Integrin binding to the matrix elicits a series of biochemical signals, such as activation of Src tyrosine kinase and focal adhesion kinase (FAK) and their downstream target effectors, activation of the Ras-extracellular signal-regulated kinase (ERK) cascade, and activation of PI3K and Rho family proteins (22, 39). These signals greatly overlap with those activated by the receptor tyrosine kinases (RTKs), which bind to soluble GFs, although the extent and duration of each signal differ depending on whether the integrin, the RTK, or both are occupied by ligand. Some signals, such as those involving Src family kinases, FAK, and Rho proteins, are predominantly activated by integrins, whereas others, including those mediated by the Ras-ERK cascade, are more dependent on activation of RTKs (23). Integrin signaling is sufficient to grant some protection from apoptosis to cells that are deprived of GFs, and to induce a motile phenotype, implying that integrin signaling is sufficient to support cell survival. In fact, integrin downstream pathways, in particular the ERK and PI3K signaling cascades, are critical points of regulation for cyclin-dependent kinases and cell-cycle progression (83). As disruption of cyclin-dependent kinase signaling can result in cell-cycle arrest, leading to apoptosis, integrin-sustained cell-cycle signaling appears to be a crucial mechanism by which adhesive receptors promote cell survival.

COOPERATION BETWEEN INTEGRINS AND GF RECEPTORS

Integrins essentially recognize positional cues encoded by the ECM and convert them into biochemical signals that control the cell response to soluble GFs and cytokines. Hence, integrins are necessary partners of growth and cytokine receptors. The current paradigm of physical collaboration between integrins and GF receptors postulates a mutual and univocal cooperation between the two signaling systems (84). Joint integrin/RTK signaling is required for cell proliferation and for optimal cell survival and cell migration. Several mechanisms

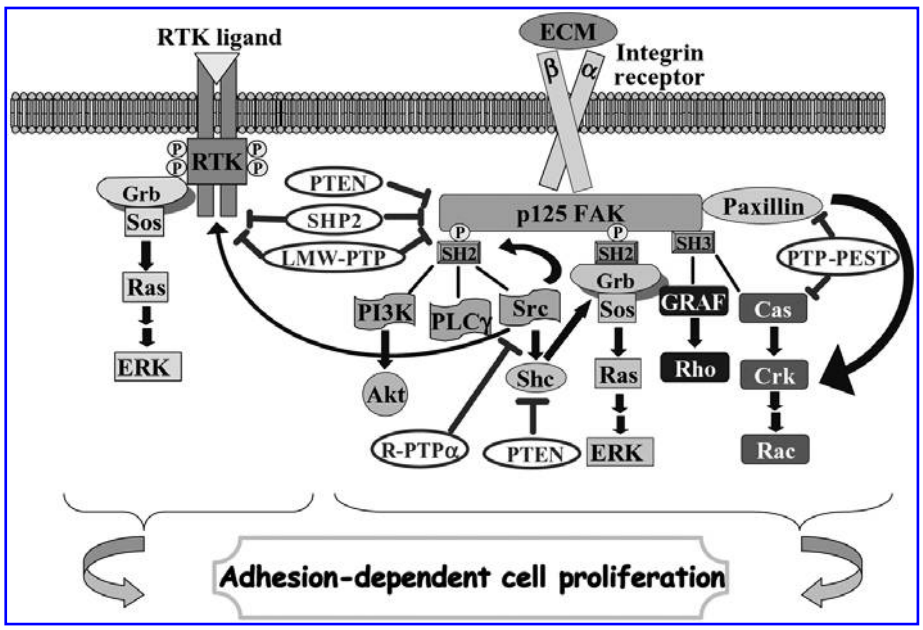

FIG. 1. The integrin signaling and the cross talk between integrins and RTKs. The model outlines the main routes arising from integrin engagement by ECM and ligand-dependent RTK stimulation. The signals triggered by both the receptors might follow parallel pathways, with additive activation of common signaling cascades. PTPs and PTKs play a pivotal role in generating a collaborative signaling between integrins and RTKs that converges, as a final response, in adhesion-dependent cell proliferation (see text for details). PLCγ, phospholipase Cγ.

ensure that the integrin and RTK signals are properly integrated by the anchorage-dependent cell. Cell adhesion is necessary to implement activation of GF receptors, and GFs are necessary to stimulate cell adhesion, migration, and the ensuing integrin-dependent signals. This results in the enhancement of GF-dependent responses, namely cell proliferation, motility, or protection from apoptosis, when cells bind the specific matrix protein that is recognized by the receptor-associated integrin. Similarly, cell detachment results in cellular desensitization to GFs, for example, the targeting of the receptor for degradation via the ubiquitin system in unadherent cells (6). This type of collaboration has been demonstrated for the αvβ3 integrin, which associates with the platelet-derived growth factor (PDGF) receptor (PDGF-R) (8, 81), the insulin and insulin-like growth factor-I receptors (81), and the vascular endothelial growth factor receptor-2 (8, 89).

More recently, some insight regarding the mechanisms regulating the nature of signaling activity of these complexes has been provided. Formation of integrin/GF-receptor complexes may lead to three kinds of signals: concerted, integrin-dependent RTK activation, and GF-dependent integrin receptor activation. In the first case, signals triggered by GF receptors and those induced by integrin engagement might follow parallel and matching pathways, with additive activation of converging signaling cascades. This type of collaboration can exploit membrane-proximal transducers, such as FAK, that act as signaling scaffolds to keep the complex together. For example, the cytosolic tyrosine kinase FAK is able to bridge the cytoplasmic tail of GF receptors and integrins through its N- and C-termini, respectively (49, 88). This coordinated activity of FAK is highlighted by the observation that fibroblasts lacking FAK are refractory to PDGF and epidermal growth factor (EGF)-dependent migration, and rescue of this defect is achieved by reexpression of a fully functional FAK able to interact with the kinase receptors and to be targeted to regions of integrin clustering (88). Accordingly, costimulation of GF receptors and integrins is necessary for efficient activation of FAK, which in turn promotes neurite outgrowth in PC12 cells (49). In the second case, integrin receptor engagement by fibronectin and/or laminin can lead to adhesion-dependent, ligand-independent activation of integrin-associated GF receptors. At least for the EGF receptor (EGF-R)-β1-integrin complex, this type of collaboration is guaranteed by Src tyrosine kinase and involves the recruitment of the adaptor protein p120Cas (66). In the last case, integrins can participate in GF-dependent signals not owing to their adhesive function. In the complex between the Met receptor for hepatocyte growth factor and α6β4, Met activation results in tyrosine phosphorylation of the integrin cytoplasmic terminal. The Met receptor/α6β4 complex, in turn, binds additional signaling molecules to amplify the signals (100).

PTKS AND PTPS INVOLVED IN ANCHORAGE CONTROL OF CELL FATE

Among integrin receptor downstream effectors are both protein tyrosine kinases (PTKs) and protein tyrosine phosphatases (PTPs). Both classes of enzymes collaborate to produce the final aim of the integrin message, namely the assembly of cell cytoskeleton and the removal of cell-cycle block. The main PTKs involved in anchorage-dependent growth are FAK, Src, and other members of its family, whereas the main PTPs retaining a role in integrin signaling are receptor protein tyrosine phosphatase α (R-PTPα), low-molecular-weight phosphotyrosine phosphatase (LMW-PTP), PTP-PEST, phosphatase and tensin homologue deleted on chromosome 10 (PTEN), and Src homology phosphatase 2 (SHP2).

pp125FAK is a 125-kDa tyrosine kinase that localizes to nascent focal adhesions, containing an N-terminal central kinase domain and a C-terminal focal adhesion targeting domain. Upon cell attachment to the ECM, FAK becomes autophosphorylated at Tyr397 either directly by integrin clustering or after phosphorylation of tyrosines 576 and 577 by Src, which enhances the catalytic activity of FAK (78). Activated FAK can bind and phosphorylate a range of different substates, which allows further recruitment of adaptor and signaling molecules. In particular, the phosphorylated Tyr397 can be bound by SH2-domain-containing proteins such as Src, Grb7, PI3K, Shc, and phospholipase Cγ. Grb2 binds the phosphorylated Tyr925. SH3-domain proteins, like p130Cas and the protein GAP for Rho associated with FAK (GRAF), interact with proline-rich sequences close to the C-terminus (45). The FAK's role in cell adhesion is complex. A dominant-negative approach implicated FAK in the process of cell spreading (74), whereas v-Src-induced FAK phosphorylation is visibly linked to focal adhesion disruption during transformation (32). Cells derived from FAK null embryos exhibit a decreased rate of migration and spreading and an increase in the number and size of peripherally localized adhesions. Previous reports have suggested that the increase in the number and size of adhesion in $Fak^{-/-}$ cells might be due to an inhibition of focal adhesion turnover (46). Taken together, these findings imply that FAK is likely to influence both focal adhesion assembly and disassembly, and is required for the dynamic regulation of integrin focal adhesions during cell migration.

The Src family of PTKs (SFKs) comprises a number of related signaling molecules, including Fyn, Yes, Lck, Blk, Lyn, Hck, Yrk, and Fgr, whose precise biological functions are poorly understood. c-Src itself is the prototype member of the family and, in addition to early descriptions of the oncogenic properties of the transforming viral oncogene v-Src in avian systems (92), mutation or perturbation in the activity or expression of c-Src is a determinative factor in cancer outcome. Indeed, c-Src is implicated in a variety of cellular processes that are linked to cancer invasion and metastasis, making it an intriguing potential target for intervention. The two best characterized tyrosine phosphorylation sites in Src perform opposing regulatory functions. The Tyr416 (in chicken Src), located within the enzyme's activation loop, undergoes autophosphorylation, which is crucial for achieving full kinase activity. By contrast, phosphorylation of the C-terminal Tyr527, mediated by the kinase Csk, inhibits Src activity. Inhibition occurs through intramolecular interactions between phosphorylated Y527 and the SH2 domain in Src, which stabilize a noncatalytic conformation. Src can also be controlled by conformational mechanisms, such as displacement of SH2/SH3-mediated interactions (24). Src kinase is activated upon ligand

Table 1. Redox Regulated PTPs and Their Role in Adhesion-Dependent Cell growth

PTPs	*Role in adhesion-dependent cell growth*	*Reference*
LMW-PTP	Regulation of PDGF-R tyrosine phosphorylation	19
	Regulation of p125FAK tyrosine phosphorylation	18
PTP-PEST	Regulation of p130Cas tyrosine phosphorylation	35
	Regulation of Csk tyrosine phosphorylation	25
	Regulation of paxillin tyrosine phosphorylation	82
SHP2	Regulation of p125FAK tyrosine phosphorylation	58, 67
	Regulation of PDGF-R tyrosine phosphorylation	50
	Regulation of Src activation through tyrosine dephosphorylation	67
	Regulation of p130Cas and paxillin tyrosine phosphorylation	59
PTEN	PdtIns(3,4,5)P_3 dephosphorylation	69
	Regulation of p125FAK tyrosine phosphorylation	41
R-PTPα	Regulation of Src activation through tyrosine dephosphorylation	104
	Regulation of p130Cas tyrosine phosphorylation	8

binding of a variety of RTKs and is involved in downstream signaling from such receptors (21). Src can also be activated by integrin-mediated ECM contact and is involved in the turnover of focal adhesions that is needed for cell migration most likely in conjunction with FAK (31). The role of Src in the process of cell migration could be the main mechanism by which its activity influences cancer-cell invasion.

An intrinsic mechanism that participates in the fine-tuning of integrin signaling is the balanced counteraction between PTKs and PTPs. Indeed, in addition to tyrosine kinases, several intracellular PTPs have been implicated as positive and negative regulators of integrin-mediated signaling.

One of the main PTPs involved in integrin signaling is R-PTPα. R-PTPα is a widely expressed transmembrane receptor PTP and has been implicated in a variety of signaling pathways. R-PTPα can associate with the SH2 domain of Grb2 upon phosphorylation of the C-terminal Tyr789, although the function of this binding is not yet clear (28). Recently, p130Cas, which is localized to focal adhesions, has been shown to be a substrate for R-PTPα (9). However, the most clearly defined substrate of R-PTPα is the PTK c-Src. R-PTPα activates Src *in vitro* and *in vivo*, and overexpression of R-PTPα leads to the activation of Src by the dephosphorylation of the C-terminal negative regulatory Tyr-527 of Src (109). R-PTPα null cells have greatly reduced Src kinase activity and are defective in cell adhesion and spreading, which are restored upon ectopic expression of R-PTPα (93). Several observations support the idea that R-PTPα activity is regulated by dimerization. R-PTPα contains a single transmembrane domain and two conserved intracellular PTP domains (D1 and D2) separated by a spacer region (104). Interestingly, the dimerization of the membrane-proximal D1 domain, which retains most if not all the activity, leads to inhibition of R-PTPα because a wedge-like structure of one monomer inserts into the catalytic cleft of the other, thereby occluding the catalytic site (90). Recent studies indicate that the second membrane-distal catalytic D2 domain of R-PTPα, which is almost completely inactive, may have a regulatory role in dimer conformation. In particular, it has been demonstrated that R-PTPα-D2 and the spacer region interact intramolecularly with each other to form a "closed" conformation, which was unable to interact intermolecularly with another monomer of R-PTPα. There is evidence that oxidative stress leads to opening up of D2, resulting in the formation of more stable, inactive dimers (93). The hydrogen peroxide (H_2O_2)-induced conformational change is dependent on the direct oxidation of Cys723 in R-PTPα-D2, suggesting a role of this residue in the observed effects.

Another intriguing phosphatase that exhibits a crucial role in integrin-mediated signal transduction is LMW-PTP. LMW-PTP is an enzyme involved in the negative regulation of PDGF-induced mitogenesis, through the direct association and dephosphorylation of the receptor (16), and in the control of cell adhesion through the regulation of the cytoskeleton reassembly (17). In particular, upon integrin engagement, LMW-PTP is able to dephosphorylate and inhibit either p190RhoGAP, thus enhancing Rho activity, or FAK, thus leading to an impairment of focal contacts formation and to an increase of cell motility (17, 75). A unique feature of LMW-PTP among all PTPs is the presence of two cysteines located in positions 12 and 17, both inside the catalytic pocket. The catalytic Cys12 is a target of oxidant species during both *in vitro* and *in vivo* exogenous oxidative stress, thus causing the inactivation of the enzyme. The additional Cys17 retains a peculiar role in the formation of an S-S intramolecular bond, which protects the catalytic Cys12 from further and irreversible oxidation and confers to LMW-PTP the ability to rapidly recover its activity (18).

A widely distributed PTP, PTP-PEST, is particularly intriguing with respect to cell motility, although its specific function remains largely unexplored. Both the overexpression of PTP-PEST in fibroblasts and its targeted deletion inhibit cell spreading and motility (37). It is unclear whether the effects on motility are due to a catalytic or scaffolding function. PTP-PEST contains an N-terminal catalytic domain and a C-terminal scaffolding domain with binding sites for numerous signaling proteins. A major physiologic substrate of PTP-PEST is p130Cas. Overexpression of PTP-PEST leads to dephosphorylation of p130Cas and disruption of its interaction with Crk. In contrast, PTP-PEST null cells contain hyperphosphorylated p130Cas, whose association with Crk is enhanced, yet these cells are unable to migrate (38). In addition

to p130Cas, PTP-PEST binds to and acts on the focal adhesion protein paxillin (86), the Src inhibitory kinase Csk (26), and the adaptor proteins Shc (12) and Grb2 (13). It is likely that PTP-PEST exerts its effects on the motility of multiple pathways through all these interactions with signaling enzymes and adaptors.

Another PTP that appears to play an important role in regulating cell adhesion and migration is PTEN. PTEN is a major and frequently mutated tumor suppressor protein. It contains a phosphatase domain with a structure resembling previously characterized PTPs, but containing an enlarged active site that can account for its ability to bind phosphatidylinositol 3,4,5-trisphosphate [PtdIns(3,4,5)P_3] and to cleave its 3'-phosphate, generating phosphatidylinositol 4,5-bisphosphate (72). PtdIns(3,4,5)P_3 is a major product of PI3K, which is activated by cell receptors, including various tyrosine kinase GF receptors and integrins (55). By antagonizing the action of PI3K, PTEN affects several cell biological processes. *In vitro*, PTEN can also remove phosphate residues from phosphotyrosine-containing peptides and proteins (97), although the relative importance of this enzymatic function *in vivo* compared with its lipid phosphatase activity has been controversial. Two cytoplasmic phosphoprotein substrates of PTEN are FAK and the adaptor protein Shc (43). However, analyses of cells from PTEN-knockout embryos fail to show changes in basal FAK phosphorylation or Shc-dependent ERK activation (57), suggesting that the major target of PTEN under steady-state conditions is PtdIns(3,4,5)P_3. Several studies have identified intriguing regulatory features in the C-terminal tail of PTEN. Mutagenesis studies demonstrate that phosphorylation of S380, T382, and T383 can modulate both the enzymatic activity and the stability of PTEN. Dephosphorylation or deletion of the tail results in enhanced phosphatase activity and rapid degradation (102). A key enzyme regulating the phosphorylation of this C-terminal cluster of serine and threonine residues appears to be the protein creatine kinase 2, which modulates PTEN stability to proteasome-mediated targeting.

Finally, the 68-kDa non–transmembrane-type PTP SHP2 has been found involved in integrin signaling. SHP2 possesses two SH2 domains in its N-terminal region (1). In response to PDGF stimulation, SHP2 binds directly to Tyr1,009 of PDGF-R through its N-terminal SH2 domain and is tyrosine-phosphorylated on Tyr542 and activated by this phosphorylation (52). SHP2 is required for integrin-evoked cell spreading, migration, and ERK activation, although the mechanism by which SHP2 mediates these effects remains unclear (47). First, SHP2 may operate in integrin signaling by regulating FAK phosphorylation. Cells deficient in SHP2 activity show FAK hyperphosphorylation, as well as increased numbers of actin stress fibers and focal contacts, as reported for FAK-deficient cells (60). Accordingly, embryos bearing an SHP2 loss-of-function mutant show gastrulation defects similar to those of FAK-knockout embryos. SHP2 may also regulate FAK function indirectly by inducing SFK activation (68). In this pathway, integrins induce weak SFK activation that results in SHP2 membrane recruitment; once there, SHP2 further activates SFK by dephosphorylation. The mechanism of SHP2-mediated cytoskeleton rearrangments is still unclear. Some reports suggest that SHP2 inhibits RhoA activity (82), whereas others propose that SHP2 positively regulates Rho activity (70). Inhibition of SHP2 also markedly increases the rate of cell attachment and cell spreading on ECM proteins such as fibronectin and vitronectin, effects that were accompanied by enhancement of adhesion-induced tyrosine phosphorylation of paxillin and p130Cas (61). These results suggest that SHP2 plays an important role in the control of cell shape by contributing to cytoskeletal organization, and that it is an important regulator of integrin-mediated cell adhesion, spreading, and migration, as well as of tyrosine phosphorylation of focal adhesion contact-associated proteins.

REACTIVE OXYGEN SPECIES AND THEIR SOURCES

Integrin signaling mostly proceeds through the involvement of adaptor proteins and enzymes regulating the phosphorylation level of other signaling proteins as stated above. Reactive oxygen species (ROS) are some of the few soluble second messengers that have been demonstrated to be essential mediators of integrins (20, 105).

ROS encompass a variety of partially reduced metabolites of oxygen (*e.g.*, superoxide anions, H_2O_2, and hydroxyl radicals) possessing higher reactivity than molecular oxygen. Inside cells, they are generated through a variety of processes, for example, as by-products of normal aerobic metabolism, or as second messengers in various signal transduction pathways, including integrin engagement (20). They can also be derived from exogenous sources, either being taken up directly by cells from the extracellular milieu, or produced as a consequence of the cell's exposure to some environmental insult. Superoxide anions are a by-product of mitochondrial electron transport, accounting for ~3% of total oxygen consumption by the organelle. The physiological activity of the respiratory chain leads to the production of semiquinones, a potential source of ROS (35). Indeed, the respiratory chain produces ROS at complex I (NADH/ubiquinone oxidoreductase) and complex III (ubiquinol/cytochrome *c* oxidoreductase). The ubiquinone site in complex III appears as the major site of mitochondrial ROS production: this site catalyzes the conversion of molecular oxygen to the superoxide anion radical ($O_2^{\bullet -}$) by a single electron transfer to molecular oxygen. Moreover, the inhibition of the respiratory chain, owing to a lack of oxygen or to an inhibitor such as cyanide or antimycin A, increases the ubisemiquinone free radical level in the normal catalytic mechanism of complex III (35).

Superoxide anions may also be synthesized enzymatically by NADPH oxidase activity, prostanoid metabolism with cyclooxygenase and lipoxygenase (LOX), xanthine oxidase, catecholamine autooxidation, and the nitric oxide (NO) synthases.

Although it remained unclear for many years whether nonphagocytic cells contain an NADPH oxidase system similar to that of phagocytes, several types of NADPH oxidases have been identified in fibroblasts and other nonphagocytic cells (27, 94). NADPH oxidase is a multiprotein complex, originally characterized in leukocytes, formed by membrane (gp91phox, p22phox) and cytosolic (Rac, p67phox, p47phox,

p40phox) proteins (3). Membrane oxidases similar to the phagocytic NADPH oxidase complex are expressed almost ubiquitously in nonphagocytic cell types (63). NADPH oxidase catalyzes the one-electron reduction of O_2 to $O_2^{\cdot -}$, which spontaneously or enzymatically dismutes to H_2O_2. Several lines of evidence demonstrate that NADPH oxidase is specifically involved in the generation of ROS by soluble GFs, such as transforming growth factor-β1 (69, 99), interleukin-1 (62), tumor necrosis factor-α (58), insulin (59), PDGF and EGF (4, 95), angiotensin II (42), thrombin, and lysophosphatidic acid (14).

5-Lipoxygenase (LOX) is a mixed function oxidase involved in the synthesis of leukotrienes from arachidonic acid (AA), and its activation is usually followed by translocation to the nuclear envelope. AA induces membrane ruffling and H_2O_2 production in a Rac1-dependent manner. Indeed, AA is generated via Rac-mediated phospholipase A2 activation in response to GFs and cytokines and is implicated in cell growth and gene expression. AA can be metabolized to leukotrienes and prostaglandins by LOX and cyclooxygenase, respectively. These AA metabolites are involved in the regulation of EGF-induced actin remodeling. Especially, leukotrienes are necessary and sufficient for the formation of actin stress fiber (87). Leukotrienes were also known to play an important role in the signaling pathway regulating cellular survival and apoptosis (87). Thus, AA is likely to act as an intracellular second messenger in the EGF-mediated signal pathway.

Transient fluctuations in ROS serve important regulatory functions, but when present at high and/or sustained levels, ROS can cause severe damage to DNA, protein, and lipids. A number of defense systems have evolved to combat the accumulation of ROS. These include various nonenzymatic molecules (*e.g.*, glutathione, vitamins A, C, and E, and flavonoids), as well as enzymatic scavengers of ROS (*e.g.*, superoxide dismutases, catalase, and glutathione peroxidase). Unfortunately, these defense mechanisms are not always adequate to counteract the production of ROS, resulting in what is termed a state of oxidative stress. Oxidative stress has been implicated in a wide variety of disease processes, including cancer, atherosclerosis, diabetes, pulmonary fibrosis, neurodegenerative disorders, and arthritis, and is believed to be a major factor in aging (34).

At the cellular level, oxidant action elicits a wide spectrum of responses ranging from proliferation to growth arrest, to senescence, to cell death. The particular outcome observed can vary significantly from one cell type to the next, as well as with respect to the agent examined, its dosage, and/or duration of treatment. However, whatever the effect seen, it largely reflects the balance between a variety of intracellular stress signaling pathways that are activated in response to the oxidative insult.

REDOX CONTROL DURING ANCHORAGE-DEPENDENT CELL GROWTH

The emerging point of view is that the balance and the duration of the oxidative burst may transform an oxidative injury in an intracellular message. Many are the observations that support the role of second messengers for ROS during anchorage-dependent cell growth. Several lines of evidence demonstrate that ROS are specifically involved in the signals elicited by soluble GFs (33). GF stimulation can induce intracellular ROS production *via* activation of the PI3K pathway, resulting in the activation of the small GTPase Rac1, which, in turn, stimulates NADPH oxidase activity (5). These findings suggest that ROS formation plays a role in mitogenic signaling elicited by cytokines and GFs.

Very recently, we provided evidence that intracellular ROS are generated following integrin engagement, and that these oxidant intermediates are necessary for integrin signaling during fibroblast adhesion and spreading (20). The increase in ROS generation with respect to suspended cells is large (~10-fold) and is dependent on integrin receptor engagement, as indicated by the growth of ROS during cell adhesion to fibronectin, but not to polylysine, and by the response elicited by anti-α5-integrin antibodies (20). In addition, the relative contribution to ROS production of integrin receptor engagement and GF administration is unbalanced, being higher in the oxidants produced during cell/ECM interaction with respect to those produced in response to GFs (Fig. 2A).

We suggest that, in parallel with the collaborative nature of integrin/RTK inside-outside signals, intracellular oxidative messages during fibroblast stimulation by mitogens may be combinatory in nature, and reflect an integrated activity of both RTKs and adhesion molecules. In fact, the rise of ROS elicited by PDGF in suspended fibroblasts is significantly less pronounced than that triggered by PDGF during simultaneous integrin receptor engagement (20) (Fig. 2B). This strongly suggests that the frequently reported generation of ROS by GFs is likely an anchorage-dependent phenomenon.

Oxidative events triggered by integrins appear mainly mediated by cytosolic LOXs (20), although an involvement of NADPH oxidase has been proposed (105). In contrast, several reports have indicated in NADPH-dependent, membrane-bound oxidases the source of GF-induced ROS (4, 18, 33, 106). Although the molecular basis of this difference is still to be elucidated, the different sources of ROS may account for the differences in both intensity and kinetics observed when redox signals elicited by GFs and by cell adhesion were compared (Fig. 2).

As other soluble second messengers, ROS act on other intracellular proteins by modulating their functions. The primary effect that ROS exert as signal transduction messengers is the reversible oxidation of proteins. Thiols, for their ability to be reversibly oxidized, are recognized as key targets of an oxidative stress (65). In addition, growing evidence indicates that thiol groups act as redox-sensitive switches, thereby providing a common trigger for a wide range of ROS-mediated signaling events. As the pK_a (where K_a is the acid constant) of the sulfhydryl group of most cysteine residues (Cys-SH) is ~8.5 and because this group is less readily oxidized by H_2O_2 than the cysteine thiolate anion (Cys-S^-), few proteins might be expected to possess a Cys-SH that is vulnerable to oxidation by H_2O_2 in cells. However, certain protein cysteine residues do exist as thiolate anions at neutral pH, as a result of the lowering of their pK_a values by charge interactions between the negatively charged thiolate and nearby positively

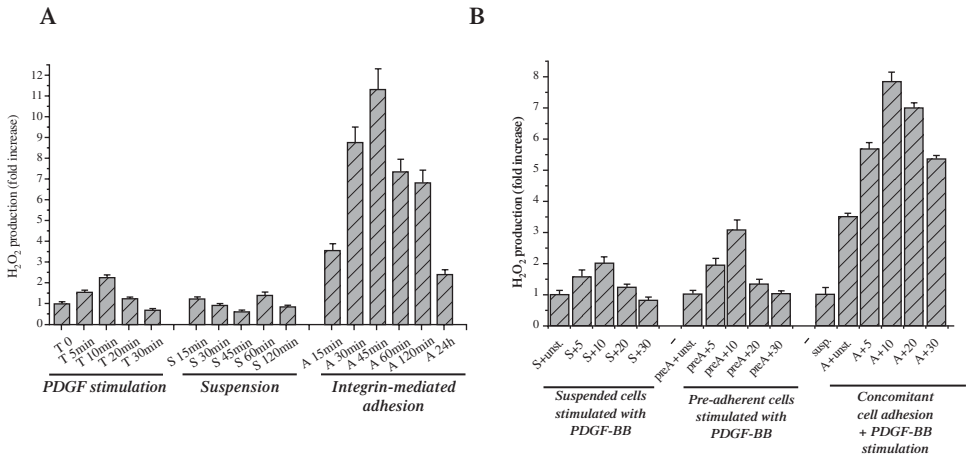

FIG. 2. Intracellular ROS level during cell adhesion. (**A**) NIH 3T3 cells (1×10^6) were serum-starved for 24 h before detaching and maintained in suspension with gentle agitation for 30 min at 37°C. Cells were then treated in suspension with 30 ng/ml PDGF-BB or plated onto fibronectin-precoated dishes for the indicated times. H_2O_2 production was evaluated with the fluorescent redox-sensitive probe 2',7'-dichlorofluorescin diacetate (DCF-DA; 5 μ*M*). (**B**) Cells were serum-deprived for 24 h and then were left adherent (preadherent cells) or detached and presuspended for 30 min. Suspended cells were either treated in suspension with 30 ng/ml PDGF-BB for the indicated times (S) or seeded onto fibronectin-treated dishes in concomitance with PDGF-BB administration (A). In parallel, preadherent cells were stimulated with 30 ng/ml PDGF-BB (preA). H_2O_2 level was evaluated with DCF-DA.

charged amino acid residues. Proteins with low-pK_a cysteine residues include PTPs, PTKs, and nuclear transcription factors (73).

A common feature of all PTPs is the presence of an essential cysteine residue (pK_a-4.7–5.4) in their active-site motif, His-Cys-X-X-Gly-X-X-Arg-Ser/Thr (where X is any amino acid), that exists as a thiolate anion at neutral pH (108). This thiolate anion accounts for the formation of a thiol-phosphate intermediate in the catalytic mechanism of PTPs. The active-site cysteine is the target of specific oxidation by various oxidant species, such as H_2O_2, and this modification can be reversed by incubation with thiol compounds such as dithiothreitol and reduced glutathione (11, 53, 54). These observations suggest that PTPs might undergo H_2O_2-dependent inactivation in cells, shifting the balanced activity between PTPs and PTKs toward protein phosphorylation. The role of ROS as second messengers is stressed by the transient nature of oxidation/inhibition of signaling enzymes. Although the formation of a cysteine sulfenic acid intermediate is highly probable for many PTPs (29), the reversibility of PTP inactivation may be guaranteed by several mechanisms that impede further degrees of oxidation of the SH group: (a) the formation of a mixed disulfide with glutathione (7); (b) the formation of an intramolecular S-S bridge (11, 18, 53, 54, 77); and (c) the formation of a sulfenyl-amide intermediate (76, 101). Indeed, the *in vivo* reversible oxidation was demonstrated first for PTP1B during EGF signaling by Lee *et al.* (53) and then for LMW-PTP during PDGF stimulation and integrin signaling due to fibronectin ligation (18, 20). The peculiar feature of LMW-PTP redox regulation was the ability to recover its catalytic activity by means of an intramolecular S-S bridge between two vicinal cysteines, both located in the catalytic site. We hypothesize that the proximity of these two cysteines in the LMW-PTP active site may confer to this phosphatase the unique ability to be rapidly regulated by the changing of intracellular redox conditions (18). More recently, Tonks and co-workers have described a reversible SHP2 oxidation during PDGF signaling (64) in an in-gel phosphatase assay screening of PTPs involved in PDGF signaling cascade. Finally, the lipid phosphatase PTEN has also been found oxidized *in vitro* and *in vivo*, its redox regulation being characterized by an intramolecular disulfur bond between very distant cysteines that leads to the reversible inhibition of the enzyme (54, 56).

H_2O_2 can also activate protein kinases and, among these, PTKs. However, the activation for some kinases appears to be due essentially to two mechanisms. First, similar to what happens for PTPs, cysteine oxidation may occur. Second, as PTKs themselves are frequently tyrosine-phosphorylated proteins and their activity is increased due to phosphorylation, most likely the concomitant inhibition of PTPs indirectly leads to sustained activation of PTKs.

Oxidation-dependent activation of PTKs may involve membrane-bound kinases, such as RTKs, intracellular kinases, such as Src tyrosine kinases, FAK, together with many other Ser-Thr or dual-specificity kinases, such as ERK or Akt and Ask (73). A direct oxidation of RTKs has been reported for insulin receptor kinase (IRK), EGF-R, PDGF-R, and Ret kinase. Schmid *et al.* have reported that optimal insulin responsiveness may require a process of "redox priming" of the β subunit of IRK, likely due to a decrease in insulin receptor β-chain sulfhydryl groups due to oxidation (79). In addition, it has been reported that "redox priming" of the IRK facilitates its autophosphorylation in the activation loop. In fact, three-dimensional models of IRK revealed that the conversion of any of the four cysteine residues 1,056, 1,138, 1,234, and 1,245 into sulfenic acid produces structural changes that bring Tyr1,158 into close contact with Asp1,083 and render

the catalytic site at Asp1,132 and Tyr1,162 more accessible (80).

The contribution of cysteine oxidation to the activation of RTK is exemplified by cRet. c-Ret is an RTK with a cadherin-like domain in its extracellular region. The c-RET proto-oncogene encodes a receptor-type tyrosine kinase, and its mutations in the germ line are responsible for the inheritance of multiple endocrine neoplasia type 2A (MEN2A) and 2B (MEN2B). Ret kinases are constitutively activated as a result of MEN2A mutations (Ret-MEN2A) or MEN2B mutations (Ret-MEN2B). The production of ROS induced by the ultraviolet (UV) irradiation of cells expressing c-Ret resulted in the dimerization of many c-Ret molecules on cell membrane (51). Dimerization was mediated by the formation of a disulfide between the Cys residues of adjacent monomers, and the dimerized receptors were preferentially autophosphorylated, resulting in their strong activation in response to ROS production during UV exposure. In particular, ROS contribute to the oncogenic potential of c-Ret as UV light irradiation induces superactivation of the constitutively activated Ret-MEN2A and Ret-MEN2B (51). It has been suggested that Ret Cys376 is one of critical target amino acids of UV irradiation for Ret kinase activation. Overexpression of Cu/Zn superoxide dismutase in cells as a result of gene transfection prevented both the UV-mediated promotion of dimerization and the superactivation of Ret-MEN2A kinase, thereby suggesting that the UV-induced free radicals in cells induce intracellular domains of Ret to dimerize the kinase proteins for superactivation.

Finally, nitration of Tyr residues in PDGF-R is observed in mild oxidant-treated cells, thus suggesting that ROS-induced modifications can occur at the GF receptor level and can be involved in the regulation of signaling pathways (30). This redox modification of PDGF-R leads to Src-dependent activation of ERK and Akt, which is independent of PDGF-R tyrosine kinase activity.

Although a specific role for RTK direct oxidation in anchorage-dependent cell growth is highly feasible, the role of their indirect redox regulation through reversible PTP oxidation in response to GF administration is well documented (15). The emerging hypothesis is that the transient negative regulation of PTPs, due to oxidants produced in response to RTK ligand stimulation, represents a strategy adopted by cells to promote RTK signaling by avoiding its prompt inactivation by PTPs. The functional relevance of ROS-mediated PTP inhibition in GF signaling has been demonstrated by blocking their accumulation. The first evidence was provided by Sundaresan *et al.* who demonstrated that overexpression of catalase in vascular smooth muscle cells blocked PDGF-R-induced tyrosine phosphorylation of ERK, as well as PDGF-induced DNA synthesis and migration (95). Second, interference with H_2O_2 production through catalase loading of A431 cells dramatically reduced tyrosine phosphorylation of EGF-R (4). Third, catalase pretreatment abolished the insulin-stimulated production of ROS, as well as the inhibition of PTP1B, and was associated with reduced tyrosine phosphorylation of the insulin receptor (59). Finally, the block of ROS production in PDGF-stimulated cells, achieved by either catalase pretreatment or inhibition of the NADPH oxidase by diphenyliodonium, leads to the reduction of PDGF-R tyrosine phosphorylation (19). In addition, during PDGF stimulation, the kinetics of ROS production, PTP redox inhibition, and receptor phosphorylation display an excellent alignment, suggesting a strict temporal correlation among these events (19). Hence, it is likely that the redox inhibition of PTPs has an important role in RTK signaling, and that the rescue (via rereduction) of the PTP catalytic activity after oxidation is followed by a dephosphorylation of activated receptor, thus terminating the signal elicited from the receptor. In this view, the ROS produced after RTK engagement may be considered as intracellular second messengers actually involved in the signal transduction machinery of soluble hormones. They lead to a feedback loop that, through the inhibition of PTPs, leads to an up-regulation of RTK tyrosine phosphorylation and ultimately to their activation.

Among intracellular PTKs, the Src tyrosine kinase and some of the members of its family are reported to be redox-regulated *in vitro*. Direct evidence of a redox-linked chemical modification of Src kinase has been obtained from *in vitro* experiments on NO-realizing agents (2). Exposure of Src to NO-releasing agents clearly promoted the catalytic activity of the kinase, either toward its autophosphorylation site or to its downstream substrates. In parallel, NO scavengers prevent its activation. The NO-induced Src activation seems to be independent of the phosphorylation on Src Tyr527, the autoinhibition site. Corresponding to this, a small portion of Src molecules are polymerized through S-S bond formation, and NO promotes this polymerization in close association with enzyme activation. The observation that the ROS-targeted c-Ret Cys residue is highly conserved in various nonreceptor PTKs, including Abl, Src, and Lck, suggests that it might also play a role in the activation of these enzymes. Consistent with this proposal, Src C-terminal residues are crucial for protein stability and cell transformation of this kinase (85).

The relevance of Src redox regulation for anchorage-dependent growth is documented by our recent data (Chiarugi and Giannoni, unpublished observations). We demonstrated that the tyrosine kinase c-Src is oxidized in response to cell attachment to the ECM and that this modification leads to an enhancement of tyrosine kinase activity and activation of downstream Src-dependent signaling (Fig. 3A and B). Nordihydroguaiaretic acid (NDGA), an inhibitor of LOX, is able to prevent the redox-dependent Src activation, in agreement with the reported ROS source during integrin signaling (20). The oxidation/activation is likely due to an S-S bond between Cys245 and Cys487, located in the SH2 and in the kinase domain of the Src molecule, respectively (Fig. 3C). We suggest that the oxidative burst after integrin engagement leads to Src kinase activation likely due to the concomitant action of direct Src oxidation and to PTP inhibition. Although our data cannot quantify the relative contribution to Src redox regulation of direct kinase oxidation or of PTP inhibition, they stress that these two phenomena are concurrent in the achievement of the full activation of the Src kinase during cell/ECM contact (Fig. 3D).

Conversely, only a few studies demonstrated the effects of ROS on FAK functions. Oxidative stress induces a decrease in the distribution of FAK in focal contacts, without alteration of the integrins in adherent trabecular meshwork (103). In a model of human umbilical vein endothelial cells subjected to

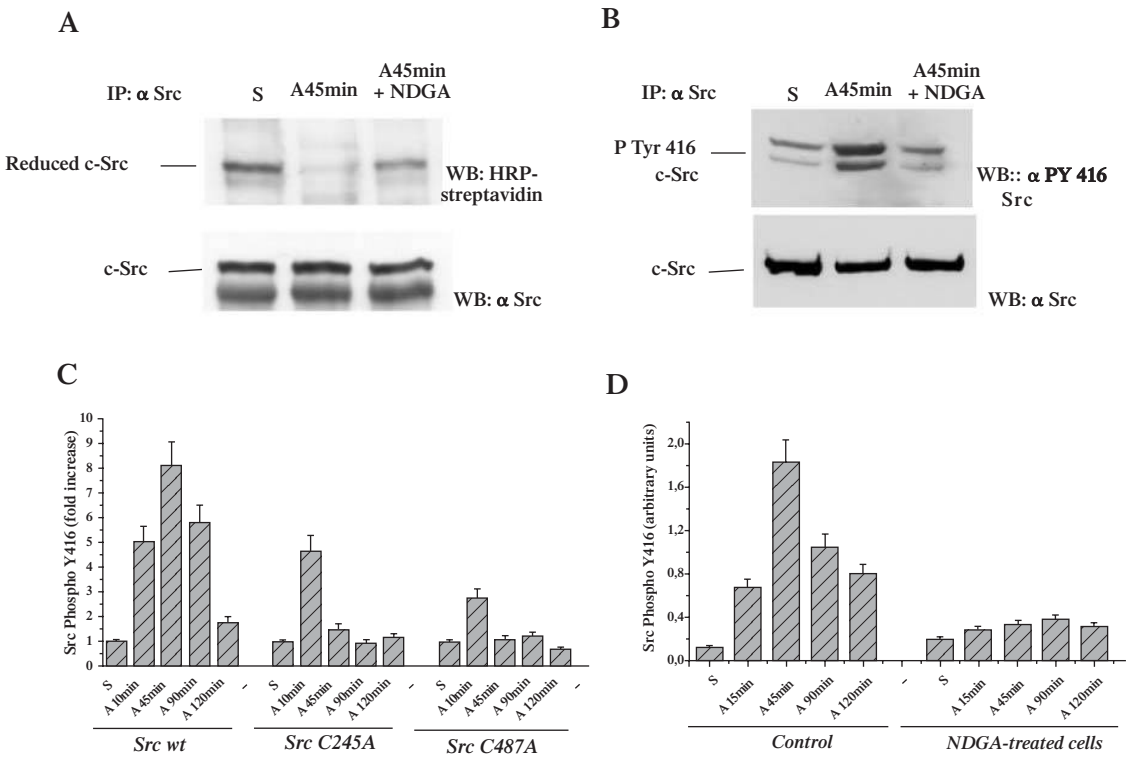

FIG. 3. c-Src is oxidized and activated during integrin-mediated cell adhesion. (**A**) NIH 3T3 cells (1×10^6) were detached, maintained in suspension for 30 min, and then seeded onto fibronectin-coated dishes with or without pretreatment with 10 μ*M* NDGA. Cell lysates were labeled with biotinyl-iodoacetyl-ethylenediamine (BIAM), an SH-specific probe, and c-Src was immunoprecipitated. An anti-horseradish peroxidase-Streptavidin immunoblotting was performed. (**B**) Cells were treated as in A, except that a Src activation was detected, by means of anti-P-Tyr416 immunoblot. (**C**) NIH 3T3 cells (1×10^6) were transfected with wild-type (wt) c-Src and with two cysteine-to-alanine oxidation-resistant mutants of the kinase (C245A and C487A, respectively). Twenty-four hours after transfection, cells were serum-starved for an additional 24 h before detaching and maintained in suspension for 30 min at 37°C. Then cells were either kept in suspension or seeded onto fibronectin-treated dishes for the indicated times. Cells were lysed and Src activation was evaluated by anti-P-Tyr416 Src antibodies. (**D**) NIH 3T3 cells (1×10^6) were detached, maintained in presuspension for 30 min, and then seeded onto fibronectin-coated dishes with or without pretreatment with 10 μ*M* NDGA for the indicated times. Cells were lysed and Src activation was evaluated as in C. In C and D, the normalized Src activation is reported.

oxidant treatment, FAK and paxillin showed an increase in tyrosine phosphorylation; at the same time, these proteins were translocated to the end of the actin stress. An increase in FAK tyrosine phosphorylation owing to oxidative stress has been described in different cells in the human glioblastoma cell line T98G (91), in human umbilical vein endothelial cells (41), in bovine pulmonary artery endothelial cells (103), and in mesangial cells (107). More recently, the molecular basis of FAK redox regulation has been described by our group. We reported that the endogenous and physiological ROS burst, occurring after integrin receptor engagement, culminated in a down-regulation of a FAK phosphatase, namely LMW-PTP (20). LMW-PTP is oxidized and inhibited in response to integrin engagement and in strict concomitance with the peak of ROS induced by ECM contact. LOX inhibitors such as NDGA, which selectively block integrin-mediated ROS generation, impede LMW-PTP oxidation/inhibition and consequently lead to FAK down-regulation. The redox regulation of FAK, through the inhibition of its dephosphorylation by LMW-PTP, leads to other key downstream events, including ERK phosphorylation, Src phosphorylation, focal adhesion formation, and cell spreading, which are all significantly attenuated by inhibition of redox signaling (20). In addition, the role of redox regulation of LMW-PTP during anchorage-dependent cell growth is further stressed by the finding of Nimnual *et al.* (67). They reported that Rac-mediated ROS production results in the down-regulation of Rho small GTPase activity, leading to Rac-induced formation of membrane ruffles during integrin-mediated cell spreading. The pathway linking generation of ROS to down-regulation of Rho involves inhibition of the LMW-PTP and then an increase in the tyrosine phosphorylation and activation of its target, p190RhoGAP. These findings, together with those concerning FAK regulation, define a key role for the redox regulation of LMW-PTP in the coupling of changes in cellular redox state to the control of actin cytoskeleton during cell adhesion and proliferation.

Finally, the redox regulation of two other PTPs may influence anchorage-dependent cell growth: SHP2 and PTEN. SHP2 has been demonstrated to be involved in PDGF-R down-regulation during mitogenic signaling through its asso-

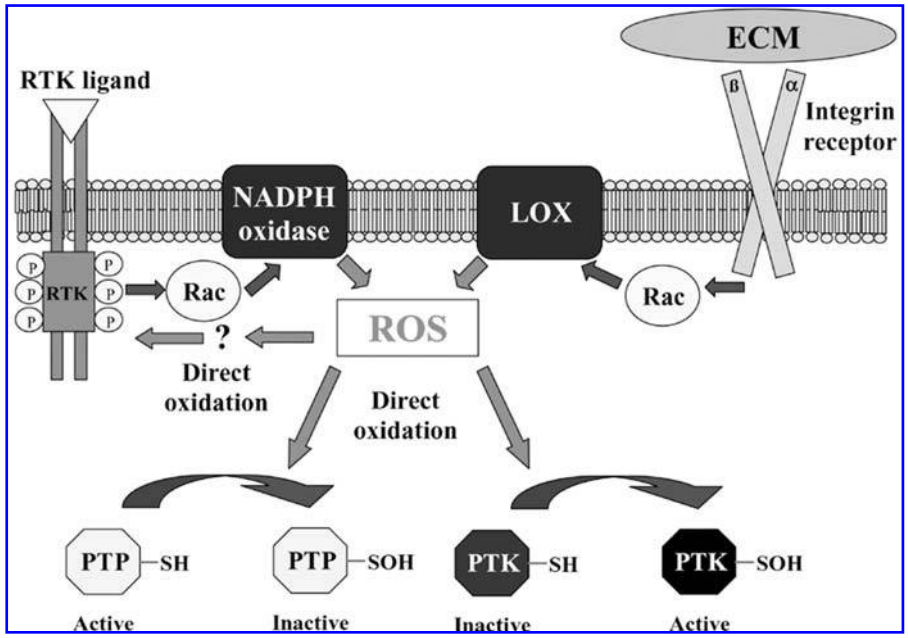

FIG. 4. Model explaining the role of ROS in anchorage-dependent cell growth. The concomitant integrin-mediated ECM contact and RTK stimulation induce the activation of 5'-LOX and NADPH oxidase, respectively, thus leading to a synergistic Rac1-dependent ROS production. ROS may act with a double approach. In the first case, ROS directly oxidize PTPs and PTKs, inducing respectively the inhibition of the phosphatases and the activation of the kinases (left side). Alternatively, ROS may be responsible for the activation of PTKs and RTKs, through the oxidative inhibition of PTPs (right side). In both cases, the cooperation between integrins and GFs promotes the phosphorylation/activation of downstream signaling and, as a final event, cell adhesion to ECM, spreading, and proliferation.

ciation with the receptor (52). SHP2 transient oxidation after ligand binding suggests a mechanism by which autophosphorylation of the PDGF-R occurs despite its association with SHP2, in agreement with the behavior of LMW-PTP in response to PDGF treatment (64). Although the role of redox regulation of SHP2 during GF stimulation is clarified, the demonstration of its oxidation during integrin-mediated cell adhesion is lacking. Furthermore, the tumor suppressor PTEN, which regulates cell migration, growth, and survival by removing the 3'-phosphate of phosphoinositides, has been found to be oxidized in response to both extracellular and intracellular oxidants (54). These results suggest that the reversible inactivation of PTEN by H_2O_2 might be important for the accumulation of 3'-phosphorylated phosphoinositides, thus contributing to cell proliferation by inhibiting PTEN function (56). Although a clear demonstration of the relevance of the redox regulation of PTEN and SHP2 during anchorage-dependent cell proliferation is warranted, it is likely that, in addition to LMW-PTP, other redox-sensitive PTPs cooperate in the regulation of the tyrosine phosphorylation of cell adhesion molecules. Both SHP2 (60) and PTEN (98) are good candidates for this role, due to their ability to dephosphorylate FAK, thus leading to cytoskeleton rearrangement. On the contrary, R-PTPα, which has been found to be oxidized *in vitro* and during UV treatment, is likely not regulated by redox signaling during cell adhesion (Chiarugi and Giannoni, unpublished observations). Indeed, R-PTPα exerts its role in the early phase of Src activation during ECM contact (10 min), whereas the peak of ROS during integrin-mediated ECM contact occurs later on (45 min), in strict concomitance with Src redox regulation.

The emerging picture concerning the role of ROS in anchorage-dependent cell growth outlined a redox circuitry whereby, upon the concomitant cell adhesion and GF stimulation, oxidative inhibition of PTPs, together with direct oxidation of some PTKs leading to their activation, promotes the phosphorylation/activation and the downstream signaling of RTK and integrin receptors and, as a final event, cell adhesion to ECM, spreading, and proliferation (Fig. 4).

ROLE OF ROS DURING ANCHORAGE-INDEPENDENT CELL GROWTH

As outlined before, in nontransformed cells, proliferation is under the coordinated control of GFs and the ECM. GF autocriny and anchorage-independent growth are observed in many transformed cells where this balance is often altered. Transformation of cells in tissue culture results in a variety of cellular changes, including alterations in serum- and adhesion-dependent cell growth, loss of contact inhibition, and changes in adhesiveness, motility, morphology, and organization of the cytoskeleton. Disruption of actin filaments and a decrease in focal adhesions are common features following transformation of cells by various oncogenes. These changes in microfilament structure are highly related to both anchorage-independent growth and cellular tumorigenicity, suggest-

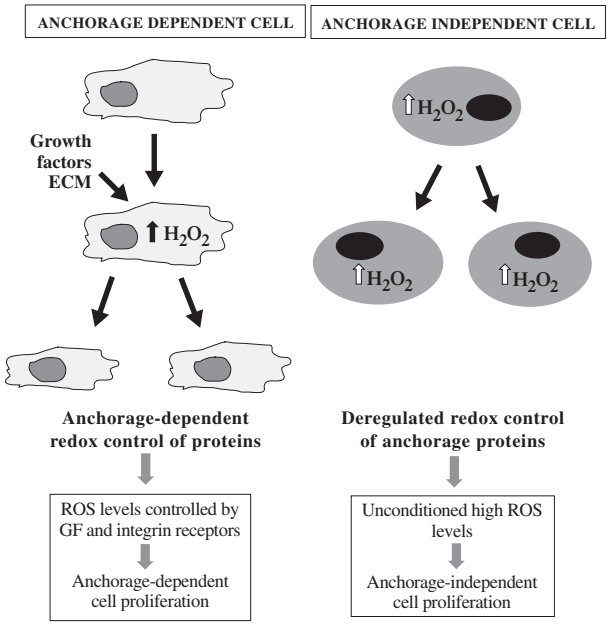

FIG. 5. The ROS outcome in anchorage-dependent and -independent cell growth. In normal cells, the concomitant stimulation of integrin and GF receptors induces a controlled ROS up-regulation, thereby maintaining an anchorage-dependent cell proliferation. Transformation of cells interferes with the right balance of ROS content, resulting in an abnormal and constant ROS production. This event coincides with the acquisition of the anchorage and GF-independent phenotype of the transformed cells.

ing fundamental roles for actin filaments in oncogenic transformation (36).

Although low levels of ROS regulate cellular signaling and play an important role in normal cell proliferation, it has been appreciated for a number of years that ROS production is increased in cancer cells (10, 96). Constant activation of transcription factors (nuclear factor-κB and activator protein-1) appears to be one functional role of elevated ROS levels during tumor progression (44). Oxidative stress can also induce DNA damage that leads to genomic instability, which may contribute to cancer progression (50). Thus, ROS are thought to play multiple roles in tumor initiation, progression, and maintenance. In this line, it has been reported that scavenging of extracellular H_2O_2 by catalase inhibits the proliferation of Her-2/Neu-transformed Rat-1 fibroblasts (71). Also, Ras mitogenic activity is, in part, superoxide-dependent (48). Compelling evidence for the transforming capacity of ROS is the finding that overexpression of Mox1 (the catalytic subunit of NADPH oxidase) induces superoxide generation and transforms NIH 3T3 cells. Furthermore, Mox1-transfected cells produce aggressive tumors in athymic mice similar in size to those produced by Ras-transformed NIH 3T3 cells (94), illustrating the critical role of this ROS regulator *in vivo*. On the basis of these considerations, it is likely that constant ROS generation during deregulated mitogenic conditions may inhibit phosphatases or activate kinases, thereby shifting the balance toward tyrosine phosphorylation of intracellular proteins, and finally the assembly of the cellular machinery to guide mitosis (15, 20). In particular, we guess that excess ROS production associated with cell transformation could release costimulatory and deregulated signals that are normally and transiently triggered by cell/ECM interaction (Fig. 5). The key role of these additional signals is underlined by the observation that constitutive overexpression of active oncogenic Rac-1 or Ras in nontransformed adherent cells confers to these cells the ability to grow in the absence of ECM contact. As expected, these cells, after acquiring anchorage-independent growth ability, display dramatic cytoskeleton architecture changes, appearing fusiform, round-shaped, highly refrangent, and displaying a lower level of cell–cell adhesion.

CONCLUDING REMARKS

In summary, the findings discussed herein clearly support the role of ROS as key second messengers driving anchorage-dependent cells to properly executed mitosis. They exert their function by regulating the activity of essential enzymes, such as PTPs and PTKs, and can, through initiation of redox-based signaling cascades, modulate cell–ECM interactions in both physiological and pathological conditions.

ABBREVIATIONS

AA, arachidonic acid; ECM, extracellular matrix; EGF, epidermal growth factor; EGF-R, EGF receptor; ERK, extracellular signal-regulated kinase; FAK, focal adhesion kinase; GF, growth factor; GRAF, GAP for Rho associated with FAK; H_2O_2, hydrogen peroxide; IRK, insulin receptor kinase; LMW-PTP, low-molecular-weight phosphotyrosine phosphatase; LOX, lipoxygenase; MEN2A and MEN2B, multiple endocrine neoplasia types 2A and 2B, respectively; NDGA, nordihydroguaiaretic acid; NO, nitric oxide; PDGF, platelet-derived growth factor; PDGF-R, PDGF receptor; PI3K, phosphatidylinositol 3-kinase; PtdIns(3,4,5)P_3, phosphatidylinositol 3,4,5-trisphosphate; PTEN, phosphatase and tensin homologue deleted on chromosome 10; PTK, protein tyrosine kinase; PTP, protein tyrosine phosphatase; ROS, reactive oxygen species; R-PTPα, receptor protein tyrosine phosphatase α; RTK, receptor tyrosine kinase; SFK, Src family of PTKs; SHP2, Src homology phosphatase 2; UV, ultraviolet.

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