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## Graphical Review

## Redox-dependent regulation of epidermal growth factor receptor signaling



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## ABSTRACT

Tyrosine phosphorylation-dependent cell signaling represents a unique feature of multicellular organisms, and is important in regulation of cell differentiation and specialized cell functions. Multicellular organisms also contain a diverse family of NADPH oxidases (NOXs) that have been closely linked with tyrosine kinase-based cell signaling and regulate tyrosine phosphorylation via reversible oxidation of cysteine residues that are highly conserved within many proteins involved in this signaling pathway. An example of redox-regulated tyrosine kinase signaling involves the epidermal growth factor receptor (EGFR), a widely studied receptor system with diverse functions in normal cell biology as well as pathologies associated with oxidative stress such as cancer. The purpose of this Graphical Redox Review is to highlight recently emerged concepts with respect to NOX-dependent regulation of this important signaling pathway.

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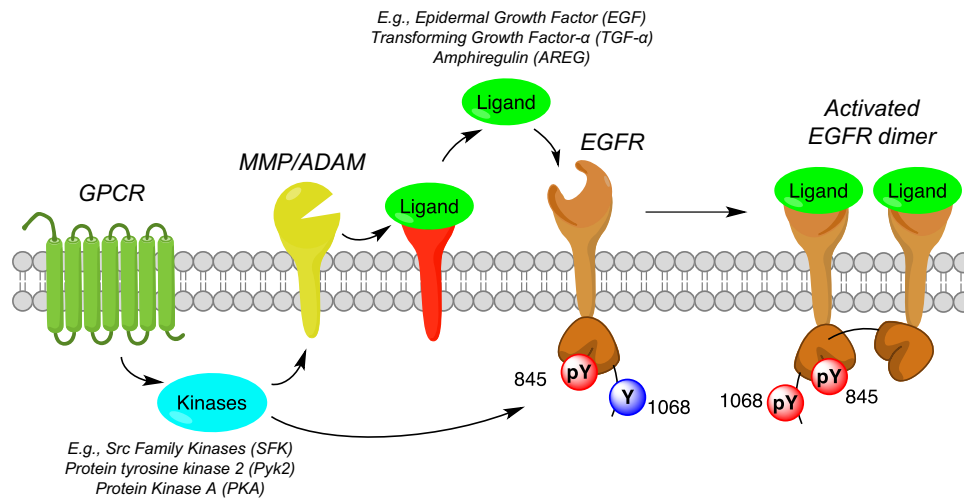
## 1. Introduction

The epidermal growth factor receptor (EGFR) is a member of the extensively studied ErbB receptor tyrosine kinase family that plays important roles in cell growth, development, differentiation, cytoskeletal organization, and cell migration. The EGFR and other ErbB members are activated by a family of ligands which promote receptor homo- or heterodimerization resulting in autophosphorylation of intracellular tyrosine residues, thereby recruiting various adapter molecules and activating an array of downstream signaling cascades [1]. In addition to this canonical activation

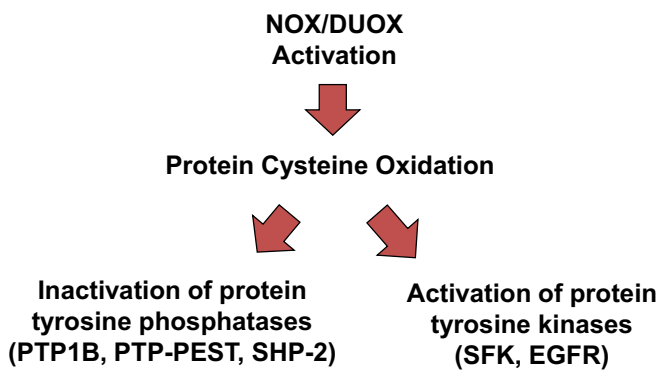
mechanism, EGFR is also frequently activated in a cross-talk mechanism by initial stimulation of G-protein coupled receptors (GPCRs), a large receptor family for diverse stimuli including proteins, peptides, lipids, amino acids, biogenic amines, and ions. Such EGFR *transactivation* occurs by two interrelated mechanisms, the first involving a “triple-membrane-passing-signal” in which GPCR activation leads to activation of membrane-bound matrix metalloproteases (MMPs) such as the ADAM (a disintegrin and metalloprotease) family, which subsequently promotes shedding of membrane-anchored EGFR ligands, allowing them to bind to ErbB receptors [2]. In addition, GPCR-dependent EGFR activation also involves ligand-independent mechanisms that result in direct EGFR phosphorylation and activation, which is mediated by intermediate activation of protein kinases that include the non-receptor Src family kinases (SFK) or protein tyrosine kinase 2 (Pyk2) (Fig. 1).

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**Fig. 1.** Direct and indirect mechanisms of EGFR activation. Activation of EGFR by cognate ligands results in homo- or heterodimerization and autophosphorylation of intracellular tyrosine residues (e.g., Y1068) to initiate cellular signaling. EGFR is commonly activated indirectly by activation of GPCR (*transactivation*), which involves intermediate activation of protein kinases such as SFK, resulting in MMP/ADAM-dependent EGFR ligand shedding as well as direct phosphorylation of EGFR (Y845) to promote EGFR kinase activation.



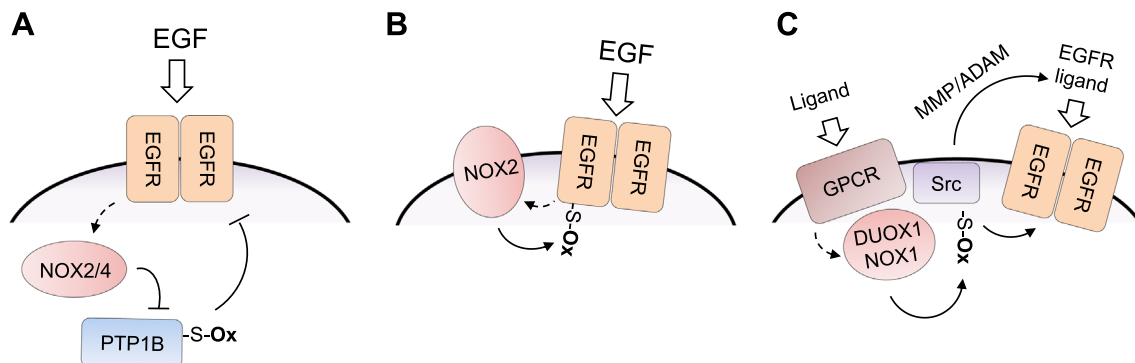
**Fig. 2.** Interrelationships between NOX/DUOX enzymes and protein tyrosine phosphorylation. Regulated activation of NOX/DUOX controls tyrosine phosphorylation by reversible oxidative modification of conserved cysteine residues with protein tyrosine phosphatases (resulting in inactivation) or in protein tyrosine kinases such as Src family kinases (SFK) or EGFR (which often enhances kinase activity).

## 2. Redox control of tyrosine kinase signaling

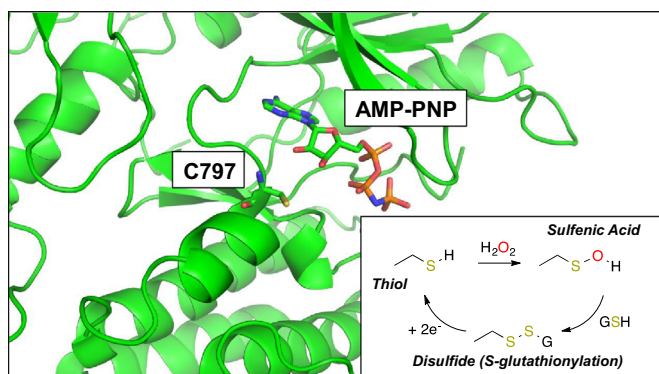
Tyrosine kinase-based signaling, including EGFR, is well-known to be subject to regulation by redox-dependent mechanisms at various levels [3] (Fig. 2). The coincidence of tyrosine

phosphorylation as an important signaling mechanism in multicellular organisms with increased diversity in NADPH oxidase (NOX) isoforms, the major source of regulated ROS production, in these more complex organisms suggests an intricate relationship in the evolution of these systems [4,5]. Pioneering studies by Rhee and co-workers illustrated that activation of EGFR is associated with ROS production, which transiently inactivates protein tyrosine phosphatases (PTPs) to enhance or prolong EGFR activation [6]. Such redox-mediated PTP inactivation is due to oxidation of a susceptible conserved catalytic cysteine residue that is essential for phosphotyrosine hydrolysis [7]. More recent studies highlighted the importance of regulated ROS production by NOX family NADPH oxidases in site-specific PTP inactivation in spatially confined areas, e.g. by NOX4 in the ER [8] or NOX2 at sites of focal adhesion [9]. Indeed, such NOX-dependent PTP inactivation is now well appreciated in promoting EGFR-dependent signaling (Fig. 3A).

In addition to the well-documented negative regulation of PTPs by oxidative mechanisms, evidence is emerging that protein tyrosine kinases are also subject to more direct redox regulation [10]. This is best studied in the SFK family, which contain a number of conserved cysteines, whose oxidation either promotes or restricts kinase activity. The EGFR, like several other protein kinases, contains a non-catalytic nucleophilic cysteine (Cys797 within EGFR), localized in the hinge region of its catalytic domain, which is



**Fig. 3.** Diverse modes of NOX dependent regulation of EGFR activation. (A) Ligand-induced EGFR activation is associated with transient cysteine oxidation (-S-Ox) within PTP1B through intermediate NOX activation (e.g. NOX2 or NOX4), resulting in its inactivation and relieving its inhibitory action of EGFR tyrosine phosphorylation and activation. (B) Ligand-induced EGFR activation results in NOX2-dependent oxidation of a conserved cysteine within the EGFR kinase domain (Cys797), which is associated with enhanced kinase activity. (C) EGFR transactivation by GPCR stimulation (e.g., P2Y<sub>2</sub>R) involves NOX activation (DUOX1 or NOX1) and cysteine oxidation within Src, which promotes Src activation and subsequent MMP/ADAM-dependent EGFR ligand shedding and as well as direct EGFR phosphorylation.



**Fig. 4.** Proposed role for reversible oxidation of C797 in EGFR kinase activation. Crystal structure of EGFR kinase domain (PDB: 3GT8) showing C797 in the hinge region within the ATP binding pocket [revealed using its analog adenosine 5'-( $\beta,\gamma$ -imido)triphosphate (AMP-PNP)]. *Inset:* Cyclic model illustrating  $\text{H}_2\text{O}_2$ -dependent thiol oxidation to sulfenic acid followed by mixed disulfide formation with GSH (S-glutathionylation) and reduction to original thiol by thiol reductases (e.g. glutaredoxin). Whereas sulfenylation of C797 may promote ATP hydrolysis, S-glutathionylation is likely to prevent ATP binding and inhibits kinase activity.

susceptible to oxidation [11]. Intriguingly, this Cys residue was found to be oxidized to a sulfenic acid ( $-\text{SOH}$ ) in response to ligand-induced EGFR activation, by recruitment and activation of NOX2 through as yet unidentified mechanisms, and was associated with increase tyrosine kinase activity [12] (Fig. 3B). Analogous sulfenylation of comparable Cys residues in other tyrosine kinases may similarly regulate kinase activity.

Recent studies by our group towards the mechanisms of EGFR activation in the airway epithelium in the context of epithelial injury or environmental allergen challenge has revealed the importance of GPCRs such as purinergic  $\text{P2Y}_2$  receptors, protease-activated receptors (PAR2), or histaminergic receptors ( $\text{H}_1\text{R}$ ), and the prominent epithelial NADPH oxidase isoform DUOX1, which critically mediates EGFR transactivation. In case of  $\text{P2Y}_2\text{R}$ -mediated EGFR transactivation, this was associated with recruitment of Src and DUOX1 to the  $\text{P2Y}_2\text{R}$  signaling complex, and cysteine oxidation within Src [13], suggesting direct targeting of Src by DUOX1-derived ROS to promote Src activation and EGFR transactivation (Fig. 3C). As another example, thrombin-mediated EGFR transactivation in smooth muscle cells involves similar redox-dependent Src activation by NOX1 [14].

Collectively, various modes of NOX-mediated oxidative EGFR regulation have been identified, which may not act independently but might act in concert to control this important signaling pathway at various distinct levels. NOX-dependent cysteine oxidation of PTP1B or EGFR itself may not be essential for EGFR activation, but rather serve to control the strength and duration of EGFR signaling, which critically determines biological outcomes. In contrast, the DUOX1-dependent oxidative activation of Src and EGFR ligand shedding is critical in initiating EGFR signaling in this transactivation pathway. More recent studies in our lab indicated that DUOX1-dependent EGFR activation involves cysteine oxidation within Src as well as EGFR and PTP1B [15,16], where the latter may involve more indirect mechanisms by other NOX isoforms or other ROS sources activated in response to initial EGFR activation to regulate the extent and duration of EGFR activation.

### 3. Proposed redox-dependent mechanism of EGFR tyrosine kinase regulation

The precise molecular mechanisms by which these distinct oxidative events control EGFR-dependent signaling is not always clear. While it is well-appreciated that oxidation of the catalytic

cysteine within PTPs results in impaired phosphatase activity, irrespective of the nature of the oxidative modification (S-nitrosylation, S-glutathionylation, sulfenyl amide formation), it is less obvious how oxidative modifications of non-catalytic cysteines in tyrosine kinases affect enzyme activity. Indeed, oxidative modification of one or more conserved cysteines in SFKs have been linked with either enhanced or reduced kinase activity, but the precise molecular mechanisms is still unclear [10,17]. Intriguingly, oxidation of Cys797 in the ATP-binding pocket of EGFR to a sulfenic acid was linked to enhanced kinase activity, potentially due to altered electrostatic or hydrogen bonding interaction with its substrate [18]. In contrast, other oxidative modifications of this Cys residue, to either S-glutathionylated adducts or to sulfonic acids, do not enhance tyrosine kinase activity [11] and might in fact be inhibitory due to steric constraints imposed by more bulky modifications of this Cys residue. Indeed, Cys797 is also the target for recently developed covalent EGFR inhibitors that have been used to treat lung cancers with activating EGFR mutations [19]. Based on these considerations, we propose that reversible oxidation of Cys797, which likely involves initial sulfenic acid formation that is subsequently resolved by S-glutathionylation and reduction, represents another level of dynamic redox-dependent EGFR regulation, complementing its regulation by tyrosine phosphorylation/dephosphorylation (Fig. 4).

### 4. Conclusion

In conclusion, current experimental evidence indicates a tight interrelationship between NOX-dependent ROS production and redox-mediated regulation of tyrosine kinases such as EGFR. The highly diverse nature of EGFR signaling in various cellular contexts is therefore not only explained by the diversity in EGFR ligands and/or variable EGFR homo- or heterodimerization, but also by variable expression and activation of diverse NOX enzymes which can control EGFR activation at various levels and thereby regulate either initiation of EGFR transactivation or the extent of EGFR activation and its diverse downstream signaling pathways. Hence, abnormalities in EGFR activation in the context of chronic diseases such as cancer or asthma may not simply be due to altered expression of EGFR or its ligands, but also be related to altered expression or activation of NOX enzymes that regulate this signaling pathway.

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