EGFR signal transactivation in cancer cells

O.M. Fischer, S. Hart, A. Gschwind¹ and A. Ullrich²
Max-Planck-Institute of Biochemistry, Department of Molecular Biology, Am Klopferspitz 18A, 82152 Martinsried, Germany

Abstract

The EGFR (epidermal growth factor receptor) plays a key role in the regulation of essential normal cellular processes and in the pathophysiology of hyperproliferative diseases such as cancer. Recent investigations have demonstrated that GPCRs (G-protein-coupled receptors) are able to utilize the EGFR as a downstream signalling partner in the generation of mitogenic signals. This cross-talk mechanism combines the broad diversity of GPCRs with the signalling capacities of the EGFR and has emerged as a general concept in a multitude of cell types. The molecular mechanisms of EGFR signal transactivation involve processing of transmembrane growth factor precursors by metalloproteases which have been recently identified as members of the ADAM (a disintegrin and metalloprotease) family of zinc-dependent proteases. Subsequently, the EGFR transmits signals to prominent downstream pathways, such as mitogen-activated protein kinases, the phosphoinositide 3-kinase/Akt pathway and modulation of ion channels. Analysis of GPCR-induced EGFR activation in more than 60 human carcinoma cell lines derived from different tissues has demonstrated the broad relevance of this signalling mechanism in cancer. Moreover, EGFR signal transactivation was linked to diverse biological processes in human cancer cells, such as cell proliferation, migration and anti-apoptosis. Together with investigations revealing the importance of this GPCR-EGFR crosstalk mechanism in cardiac hypertrophy, Helicobacter pylori-induced pathophysiological processes and cystic fibrosis, these findings support an important role for GPCR ligand-dependent EGFR signal transactivation in diverse pathophysiological disorders.

Introduction

Cellular signal transduction networks serve to co-ordinate the plethora of extracellular stimuli into biological responses of the cell. Within these communication networks, receptor cross-talk has emerged as a general signalling mechanism combining and diversifying signal transduction pathways. Key components of these networks are cell-surface receptors which transduce external signals through the membrane into the cell. Receptor tyrosine kinases represent an important subclass of these transmembrane proteins, with the EGFR (epidermal growth factor receptor) being the most prominent representative. The EGFR controls a wide variety of biological responses such as proliferation, differentiation, migration and the modulation of apoptosis. Aberrant receptor signalling due to overexpression, mutation or autocrine signalling loops has been frequently implicated in hyperproliferative diseases such as cancer.

Direct stimulation of the receptor by binding of a ligand to the receptor’s extracellular domain leads to dimerization and subsequent autophosphorylation of two receptor molecules, thereby creating phosphotyrosine docking sites to activate intracellular signalling cascades. Eight ligands have been described so far for the EGFR: EGF (epidermal growth factor), HB-EGF (heparin-binding EGF), amphiregulin, TGF-α (transforming growth factor α), betacellulin, epiregulin, epigen and crypto. All of them, except crypto, are synthesized as membrane-spanning precursor molecules that have to be proteolytically processed to become fully active.

Several modes of indirect EGFR activation have been described. Stimulation of EGFR phosphorylation occurs after treatment with unphysiological stimuli, including hyperosmolarity, oxidative stress, mechanical stress, UV light and γ-irradiation. This effect has been predominantly attributed to the inactivation of phosphatases that antagonize the intrinsic receptor kinase activity, thereby shifting the equilibrium of basal autophosphorylation and dephosphorylation towards the activated state. Apart from unphysiological stimuli, receptor activation can also be induced by chemokines, cell-adhesion molecules and GPCRs (G-protein-coupled receptors).

GPCRs represent the largest group of cell-surface receptors and exert a wide variety of biological function, including neurotransmission, photoreception, chemoreception, metabolism, growth, differentiation and migration [1]. The finding that GPCR stimulation induces EGFR phosphorylation combines the broad diversity of GPCRs with the potent signalling capacities of the EGFR and serves as a paradigm for inter-receptor cross-talk. Since increasing evidence implicates this so-called EGFR signal transactivation in diverse pathophysiological disorders, elucidation of the underlying mechanism combining and diversifying signal transduction pathways has been frequently implicated in hyperproliferative diseases such as cancer.
signalling mechanisms will help us to understand the highly complex network of signal transduction pathways and the relevance of its dysfunction in human disorders.

**GPCR-induced EGFR signal transactivation**

EGFR signal transactivation by GPCRs was originally described by Daub and colleagues [2]. Treatment of rat fibroblasts with LPA (lysophosphatidic acid), ET-1 (endothelin-1) or thrombin leads to rapid transient EGFR phosphorylation and subsequent activation of downstream signalling events such as MAPK (mitogen-activated protein kinase) phosphorylation or c-fos gene expression. These signalling events critically depend on EGFR function, since both the specific EGFR kinase inhibitor AG1478 and a dominant-negative EGFR mutant abrogate this GPCR-induced signalling. Thereafter, various studies demonstrated that GPCR-induced EGFR signal transactivation occurs in a variety of cell types, including vascular smooth muscle cells, human keratinocytes, primary mouse astrocytes and PC12 cells [3–5].

Interestingly, different classes of G-proteins have been shown to be involved in this transactivation process, including G<sub>i</sub>, G<sub>q</sub> and G<sub>13</sub> proteins, although to date there have been no data available implicating G<sub>i</sub>-coupled receptors in EGFR signal transactivation [6,7].

**Intracellular mediators of EGFR signal transactivation**

Since no EGF-like ligands could be detected in conditioned cell-culture media of GPCR ligand-stimulated cells, the transactivation mechanism was attributed to a ligand-independent and therefore intracellular mechanism. Different cytosolic signal transduction proteins have been implicated in the transactivation process.

Src-family tyrosine kinases have been suggested as both upstream and downstream mediators of the EGFR in GPCR-induced transactivation. Inhibitor studies suggested that the presence of Src-family kinases upstream of the receptor in vascular smooth muscle cells [8], in immortalized hypothalamic neurons [9] and in LPA-stimulated COS-7 cells [10]. In contrast, other studies showed EGFR phosphorylation to be independent of Src activity in COS-7 and HEK-293 cells [3,11,12]. Several other reports demonstrated GPCR-induced MAPK activation to depend on both EGFR and Src activity [13,14]. Together, these reports did not clearly depict the role of Src within the EGFR signal transactivation process, whether acting as an upstream or downstream signalling partner of the EGFR.

Besides Src kinases, the serine/threonine kinase PKC (protein kinase C) has been frequently shown to be involved in EGFR signal transactivation. In cardiomyocytes, PC-12 and HEK-293 cells EGFR signal transactivation was shown to be strictly dependent on PKC activity [15–17]. Moreover, in different cellular systems such as vascular smooth muscle cells, cardiac fibroblasts, PC-12 cells and ovarian carcinoma cells the intracellular Ca<sup>2+</sup> concentration has been reported to be critical for EGFR signal transactivation [4,5,16,18]. Within this context, the Ca<sup>2+</sup>-regulated tyrosine kinase Pyk2 has been discussed as a mediator of EGFR signal transactivation [16,19]. However, inducible expression of a kinase-inactive Pyk2 mutant did not affect bradykinin-induced calcium-dependent EGFR phosphorylation in PC-12 cells [7], and inhibitor studies in ET-1-stimulated rat cardiomyocytes did not reveal a functional interdependence of either EGFR or Pyk2 activity, suggesting a role for Pyk2 in parallel to the EGFR.

Together, these reports suggest several cytoplasmic signalling proteins as mediators of the EGFR signal transactivation pathway depending on the cellular system and the signalling context. However, recent results implicating EGF-like ligands in the GPCR-induced transactivation process have shed new light on the molecular mechanisms underlying GPCR-induced EGFR activation and provide a convergence point for intracellular signalling proteins within the GPCR–EGFR cross-talk mechanism.

**EGFR signal transactivation involves metalloprotease-mediated EGF-like ligand shedding**

Although different investigations suggested a ligand-independent intracellular signalling mechanism [3,5,17], Prenzel and colleagues [20] were the first to demonstrate the metalloprotease-mediated processing of the EGF-like ligand HB-EGF and therefore a ligand-dependent mechanism in EGFR signal transactivation. Using a chimaeric receptor tyrosine kinase they provided evidence for the involvement of the extracellular ligand-binding domain of the EGFR in the GPCR-induced transactivation pathway. Blocking both proHB-EGF function using the diphtheria toxin mutant Crm197 and metalloprotease function with batimastat (BB-94) aborted GPCR-stimulated EGFR, Shc and MAPK phosphorylation, revealing the involvement of zinc-dependent metalloproteases and the EGF-like ligand HB-EGF in the transactivation pathway in COS-7, HEK-293 and Rat-1 cells. Since processed HB-EGF is retained in the heparan sulphate proteoglycan matrix, the released ligand could not be detected in the cell culture supernatant. These findings led to the model of the TMPS (triple membrane-passing signal; Figure 1). GPCR stimulation leads to activation of a metalloprotease, resulting in proHB-EGF processing. Subsequent release of the mature growth factor activates the EGFR and its downstream signalling cascades. Since this initial discovery, many reports have revealed the broad relevance of this signalling mechanism within a variety of cellular systems, including the thrombin-induced EGFR transactivation and cell migration of smooth muscle cells [21], EGFR activation following *Helicobacter pylori* infection in gastric epithelial cells [22,23], isoprenaline stimulation of cardiac fibroblasts [24] and stimulation of renal proximal tubule cells with 14,15-epoxyeicosatrienoic acid [25].

Very recent studies have identified the metalloproteases involved in the EGFR signal transactivation process as...
Mitogenic and Migratory Signals from GPCRs and Tyrosine Kinases

Figure 1 | TMPS mechanism of EGFR transactivation

GPCR stimulation leads to metalloprotease-dependent processing of EGF-like ligands, which in turn activate the EGFR and downstream signalling cascades.

members of the ADAM (a disintegrin and metalloprotease) family of zinc-dependent metalloproteases. Lemjabbar and Basbaum [26] demonstrated the involvement of ADAM10 in the bacterial lipoteichoic acid-induced activation of the platelet-activating factor receptor, leading to proHB-EGF shedding and EGFR signal transactivation in the lung carcinoma cell line NCI-H292. ADAM10 was further implicated in EGFR transactivation in COS-7 and PC-3 cells as a mediator of bombesin-induced proHB-EGF shedding [27]. In contrast Asakura et al. [28] implicated ADAM12 in processing of proHB-EGF in GPCR-induced cardiac hypertrophy in cardiomyocytes after stimulation with vasoactive catecholamines and peptides, including phenylephrine, angiotensin II and ET-1 [28]. Gschwind and colleagues [29] demonstrated processing of pro-amphiregulin by ADAM17 [TACE (tumour necrosis factor-α-converting enzyme)] in head and neck squamous cell carcinoma (HNSCC) after stimulation with LPA and carbachol [29], leading to EGFR phosphorylation, activation of MAPKs and Akt. Moreover this TACE- and amphiregulin-dependent EGFR signal transactivation could be linked to biological responses such as cell proliferation and migration. ADAM17-mediated release of amphiregulin has also been reported in response to stimulation of the lung carcinoma cell line NCI-H292 with cigarette smoke [30]. Interestingly, this report demonstrated the occurrence of two distinct ligand-dependent pathways leading to EGFR signal transactivation in the same cellular system, one involving ADAM10 and proHB-EGF processing [26], and the other one utilizing ADAM17 and pro-amphiregulin release.

Apart from HB-EGF and amphiregulin, TGF-α has also been related to EGFR signal transactivation and subsequent MAPK activation in T84 cells in response to carbachol [31] and in gastric epithelial cells after prostaglandin E2 treatment, but the metalloprotease responsible has not been identified [32]. Interestingly, co-stimulation of metabotropic and AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolpropionic acid) receptors on hypothalamic astrocytes has been shown to lead to metalloprotease-mediated transactivation of the EGFR, presumably involving TGF-α [33]. This mechanism suggests a functional link between the glutamatergic neuronal system and the astrocytic EGFR signalling in the neuroendocrine brain in a neuron-to-glia signalling pathway, which is potentially involved in mammalian sexual development.

Members of the ADAM family of zinc-dependent metalloproteases have been frequently implicated in the processing of proEGF-like ligands and various other transmembrane proteins [34]. Studies using fibroblasts derived from TACE-knockout mice implicated ADAM17 in the release of TGF-α and other EGF-like ligands as well as in the constitutive availability of these growth factors [35,36]. Furthermore, ADAM9 has been shown to release proHB-EGF after treatment of VeroH cells with PMA [37], while in the same
Table 1 | Critical elements of TMPS pathways

<table>
<thead>
<tr>
<th>Cell line or tissue</th>
<th>Stimulus</th>
<th>GPCR</th>
<th>Protease</th>
<th>EGF-like ligand</th>
<th>Biological response</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>HNSCC</td>
<td>LPA</td>
<td>Edg</td>
<td>ADAM17</td>
<td>Amphiрегulin</td>
<td>Proliferation, cell-cycle progression, migration</td>
<td>[29,46]</td>
</tr>
<tr>
<td>Lung epithelia, NCI-H292</td>
<td>Gram-positive lipoteichoic acid</td>
<td>PAFR</td>
<td>ADAM10</td>
<td>HB-EGF</td>
<td>Ras, mucin synthesis</td>
<td>[26]</td>
</tr>
<tr>
<td>Rat neonatal cardiomyocytes</td>
<td>Phenylephrine, angiotensin II, ET-1</td>
<td>AngR, ETR</td>
<td>ADAM12</td>
<td>HB-EGF</td>
<td>Cardiac hypertrophy</td>
<td>[28]</td>
</tr>
<tr>
<td>COS-7, PC-3</td>
<td>Bombesin</td>
<td>BombR</td>
<td>ADAM10</td>
<td>HB-EGF</td>
<td>SHC, Gab1, ERK</td>
<td>[27]</td>
</tr>
<tr>
<td>Murine fibroblasts</td>
<td>PMA</td>
<td>-</td>
<td>ADAM9</td>
<td>HB-EGF</td>
<td>ProHB-EGF shedding</td>
<td>[37]</td>
</tr>
<tr>
<td>Gastric epithelia, colon cancer</td>
<td>Prostaglandin E2</td>
<td>-</td>
<td>ADAM17</td>
<td>HB-EGF</td>
<td>ProHB-EGF shedding</td>
<td>[35]</td>
</tr>
<tr>
<td>T-84</td>
<td>Carbachol</td>
<td>Muscarinic AChR</td>
<td>?</td>
<td>TGF-α</td>
<td>ERK, c-Fos, proliferation</td>
<td>[31]</td>
</tr>
<tr>
<td>MKN-1, ST42, MKN-28</td>
<td>Helicobacter pylori</td>
<td>?</td>
<td>?</td>
<td>HB-EGF</td>
<td>Potentially involved in neoplastic transformation</td>
<td>[23]</td>
</tr>
</tbody>
</table>

cellular system LPA-stimulated processing of proHB-EGF is independent of ADAM9 [38], suggesting that proHB-EGF sheddases are defined by both the cellular context and the stimulus. Together with the investigation by Lemjabbar et al. [30] reporting that in the same cellular system different protease/ligand combinations can act as transducers of GPCR-induced EGFR stimulation, these findings provide evidence for the high diversity of signalling components employed in EGFR signal transactivation. Table 1 provides a short overview of these critical components in the transactivation process.

So far the identity of the signalling elements in between the GPCR and the ADAM proteases has remained elusive. Mechanistic studies on the regulation of cell-surface shedding events implicated the MAPKs ERK (extracellular-signal-regulated kinase) 1 and 2 in the processing of TGF-α upon treatment with platelet-derived growth factor, fibroblast growth factor or EGF, while p38 was shown to control basal TGF-α shedding [39]. Moreover, different reports demonstrated phosphorylation of the intracellular domain of ADAM17 in response to PMA and growth factors [40,41]. In contrast, Black and co-workers [42] reported that the cytoplasmic tail of ADAM17 is dispensable for PMA-stimulated release of tumour necrosis factor-α and other substrates.

Taken together, the elucidation of the ligand-dependent EGFR signal transactivation mechanism has provided a new and general signalling scheme for inter-receptor cross-talk which has been implicated in a wide variety of diverse cellular systems and signalling contexts. Still, the signalling events regulating the shedding processes that lead to the release of EGF-like ligands are poorly defined, and future efforts will have to focus on their characterization. In particular, these signalling components provide a link to autocrine growth factor loops involving the EGFR and its downstream signalling components of the MAPK family initiated by autocrine or paracrine GPCR-ligand stimulation.

Ligand-dependent EGFR signal transactivation in cancer

Aberrant EGFR activation due to overexpression, mutation and autocrine growth factor loops has been frequently related to hyperproliferative diseases such as cancer. The discovery of the ligand-dependent EGFR signal transactivation pathway provides a new mechanistic concept in cancer development and progression, as autocrine signalling loops involving GPCR ligands are likely to contribute to and drive autocrine EGFR stimulatory mechanisms. Analysis of GPCR-induced EGFR phosphorylation and downstream signalling in more than 60 human carcinoma cell lines derived from different tissues revealed the broad occurrence of this signalling mechanism in cancer (N. Prenzel, E. Zwick, A. Gschwind, S. Hart, M. Leserer, B. Schäfer, O.M. Fischer, M. Buschbeck, M. Gensler and A. Ullrich, unpublished work), and an increasing number of publications emphasize these findings. In the prostate carcinoma cell line PC-3, bombesin- and PMA-stimulated EGFR activation has been reported to occur in a ligand-dependent manner, while blocking metalloprotease function in unstarved PC-3 cells diminished high basal EGFR phosphorylation [20]. Furthermore, LPA treatment of PC-3 cells induces metalloprotease-dependent EGFR and ERK activation [44], while bradykinin-induced EGFR transactivation enhanced cell proliferation [45]. Recently, Gschwind et al. [29,46] demonstrated LPA-induced EGFR signal transactivation in head and neck squamous cell carcinoma which was linked to cell proliferation, cell-cycle progression and increased migration...
involving ADAM17 and amphiregulin. Moreover, ligand-dependent EGFR and ERK phosphorylation in response to GPCR stimulation was shown in colon cancer cells after carbachol treatment leading to cell proliferation [47], in the ovarian carcinoma cell line Sk-Ov-3 in response to interleukin-8 [18] and in U-373 MG glioblastoma cells after stimulation with substance P resulting in increased biosynthesis of the transcription factor Egr-1 [48]. Interestingly, H. pylori-induced inflammatory processes and its role in human gastric carcinogenesis have been linked to HB-EGF-dependent EGFR signal transactivation in human gastric epithelial tumour cells [22,23], and gastrin-CCK(B) cholecystokinin B) receptor stimulated ligand-dependent EGFR phosphorylation is involved in enhanced cell migration of gastric epithelial cells [49]. A recent publication by Givenni and colleagues [50] demonstrated metalloprotease-mediated EGFR signal transactivation in response to Wnt expression in mammary epithelial cells, thereby proposing a new mechanistic basis for Wnt-induced oncogenesis. Furthermore, a recent publication by Lemjabbar and colleagues [30] linked EGFR signal transactivation to increased cell proliferation in human lung carcinoma cells in response to cigarette smoke, implicating EGFR signal transactivation in the development and progression of lung cancer of cigarette smokers.

Conclusions

Tremendous efforts have been achieved into the elucidation of the molecular mechanisms of inter-receptor cross-talk. EGFR signal transactivation serves as the paradigm for such cell-surface receptor cross-communication, changing the classical view of linear signalling cascades towards a highly interconnected complex signal transduction network.

Within the EGF-like ligand-dependent EGFR signal transactivation mechanism, different metalloproteases of the ADAM family (namely ADAM10, 12 and 17) as well as different EGF-like ligands (HB-EGF, TGF-α and amphiregulin) have been recently identified. The finding that in the same cellular system different proteases in combination with the same or a different EGFR ligand are capable of inducing EGFR signal transactivation demonstrates the diverse complexity of this signal transduction system. The broad relevance of EGFR signal transactivation in the development and progression of human cancer has been demonstrated and extended towards other pathophysiological diseases, including cystic fibrosis [26] and cardiovascular diseases [28]. Future investigations will have to focus on the regulation of metalloprotease activity in response to GPCR stimulation. As compelling evidence demonstrates the significance of EGFR signal transactivation in human disorders, the components of this signalling mechanism represent promising targets for therapeutic intervention.

O.M.F. has been supported by the Boehringer Ingelheim Fonds.

References


©2003 Biochemical Society
43 Reference deleted

Received 6 June 2003