STRUCTURE AND ACTIVATION OF EGF RECEPTOR: MINIREVIEW

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Epidermal growth factor receptor plays crucial role through the development, controls cell proliferation, differentiation and survival, and gen activation. Inappropriate activation of the receptor is associated with neoplastic transformation of many cell types. EGFr is a member of growth factor receptor tyrosine kinase family: EGFr/ErbB-1, HER2/ErbB-2, HER3/ErbB-3 and HER4/ErbB-4 activated by a range of growth factors. EGFr is activated by six ligands: epidermal growth factor (EGF), transforming growth factor α (TGFα), amphiregulin, heparin-binding EGF-like growth factor, betacellulin and epiregulin. These receptors with their ligands are considered to be important in the development, progression and behavior of human cancers.

Structure of EGF receptor

The human epidermal growth factor receptor (EGFr) is a 170 kDa transmembrane glycoprotein, consist of (Fig. 1):

Extracellular domain of 621 amino acids (AA) contains binding site for ligand. This domain comprises of four regions. Region I consist of amino acids 1-165 and is important in ligand induced dimerization (FERNANDES et al. 2001). Region II (AA 166-309) and IV (AA 482-621) are rich in cysteins (ABE et al. 1998), which are linked by intra-chain disulfide bonding. Region III comprises amino acids 310-481 and represents the binding site for EGF.

Intracellular domain of 541 AA consists of tyrosine kinase region and the C-terminal tail with five autophosphorylation sites (Tyr992, Tyr1068, Tyr1086, Tyr1148 and Tyr1173) (FERNANDES et al. 2001). These two domains are conected by hydrophobic *transmembrane* single spanning *domain* of 24 AA.

Though EGFr is a transmembrane receptor, recently it has been detected in the nucleus in many tissues and cell lines. The potential function of nuclear EGFr was unclear. Recent data indicate the function of EGFr as a transcription factor for activation genes involved in proliferating processes (LIN et al. 2001; MARTI et al. 2001; WONG and CHAN 2001).

The knowledge of receptor activation processes is substantial for understanding the role of the receptors themselves. This would provide the means for efficient inhibition of the receptor intracellular signalling in situations of uncontrolled activity like for anticancer therapies. Till today, two contrary models of EGFr structure exist. Nevertheless the crucial discrepancy in holo receptor configuration exists, the common characteristic for both is the activation of tyrosine kinase by ligand binding.

The first model is based on monomeric character of naturally occuring EGFr under unstimulated condition. Binding of ligand to its EGFr induces dimerization of two molecules of the monomeric receptor-ligand complex forming dimeric complex. This process iniciates kinase activation, e.g. phosphorylation of its own tyrosine residues (autophosphorylation) on C-terminus of intracellular domain via inter-molecular cross-phosphorylation. The amino acid sequences containing phosphorylated tyrosine residues serve as binding sites for cytoplasmic proteins.

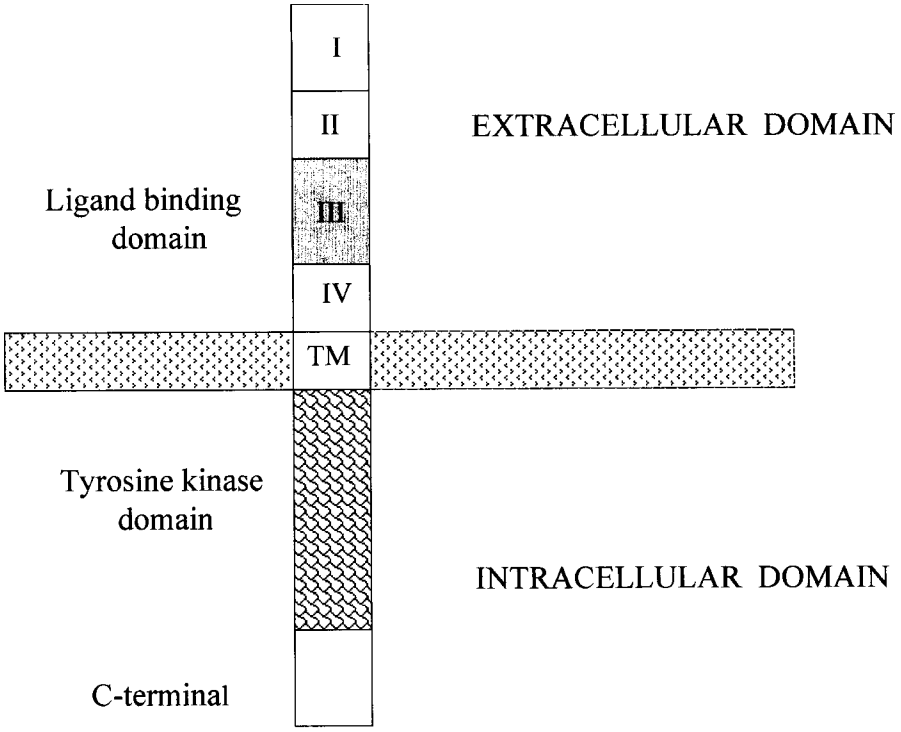

Fig. 1 Structure of the EGF receptor. (TM – transmembrane domain).

Some of these proteins possess catalytical activity, whereas others serve as adaptors that couple receptors and enzymes involved in signal transduction (Sorkin 2001). The subsequent dissociation of these signaling complexes releases activated effector and adapted proteins into cytoplasm where they stimulate many different signal transduction cascades, such as the mitogen-activated protein kinase (MAPK) pathway, phosphoinositol kinase, the antiapoptotic kinase Akt and several transcriptional regulators. The enzymatic cascade culminates in gene activation and cellular response (Yarden 2001).

The model of EGFr dimerization in the process of receptor activation is further subdivided into two mechanisms: 1/ The irreversible model is based on irreversible conformational changes of EGFr upon EGF binding (Canals 1992), while 2/ the reversible model suggests that EGFr would be in fast equilibrium between monomer and dimer, and binding of EGF would shift the equilibrium toward dimer formation (Zhou et al. 1993; Sherrill and Kyte 1996). Previous hypothesis considered symmetric process of EGFr dimerization, e.g. EGF binds to EGFr monomer and the EGF-EGFr complex will form homodimer (Sherrill and Kyte 1996; Lemmon et al. 1997). However, recent studies suggested an asymetric dimerization process: EGF binds to EGFr and this EGF-occupied EGFr will prefer to form heterodimer with another monomer before the binding of the second EGF (Sako et al. 2000). Only dimers occupied with two molecules of EGF are kinase active (Lemmon et al. 1997; Sako et al. 2000). Kinetic analysis of the dimerization and kinase activity suggested that dimerization of EGFr is prior to the activation of tyrosine kinase (Sherrill 1997). Most recent is the proposal of symetric two-step activation mechanism for EGFr tyrosine kinase. 1/ Binding of EGF to EGFr forms dimers; conformational change of the extracellular EGF-binding domain is transmitted to the intracellular part and leads to increase of the affinity for ATP. 2/ ATP binding to the kinase domain facilitates the interaction of intracellular domains resulting in increase of kinase activity (Ge et al. 2002).

Mature EGFr is a glycoprotein. Glycosylation by N-linked oligosacharides of EGFr is important for ligand binding induced activity (Slieker et al. 1986). Glycosylation induces conformational changes resulting in proper folding of the full-length receptor

and thus creating EGF-binding active conformation. (Fernandes, 2001). Glycosylation in region III of extracellular domain of EGFr is probably sufficient to induce receptor-active conformation i.e. a conformation needed for EGF binding, what means that glycosylation positively regulates receptor-receptor association (Fernandes, 2001). It was also found, that complete removal of carbohydrate chains from glycosylated EGFr neither abolished the ligand binding activity, nor the kinase activation (Fernandes, 2001) what indicate, that the initial N-glycosylation is sufficient for kinase activation. With respect to these observation a new detailed model for EGF binding and receptor activation was proposed (Fernandes, 2001). Mature receptor is in a monomeric state under the negative influence of region I in extracellular domain, which is stronger that positive influence exerted by region III. EGF binding to region III somehow overcomes the inhibitory effect of region I. Thus activation of the receptor after ligand binding could reflect the ability of a ligand to remove negative constrain.

The second model suggests that the EGFr under basal (nonstimulated) conditions exists as preformed dimeric structure on the cell surface in vivo. EGFr dimers stabilized via cystein residue cross linking are not active. Restricted kinase activity may result from restricted flexibility of the kinase. Activation of the receptor iniciated by ligand binding induces rotation of juxtamembrane region of EGFr, hence the transmembrane domain, which dissociate the cytoplasmic domains and results in activation of the kinase. Moriki et al. (2001) describe that ~70 % of total EGFr receptors and ~80 % of cell surface EGFr receptors are present as dimers at 37 °C. Proposed „Flexible rotation" model of EGFr activation consists of following steps: 1/ EGFr on the cell surface exists as an inactive dimer with flexible ligand-binding domain and relatively stable juxtamembrane domain. 2/ Upon EGF binding the juxtamembrane domain flexibly rotate or twist in parallel with the plane of the cytoplasmic domain. EGF is likely to stabilize the flexible extracellular domains and induce rotation of the juxtamembrane regions. Binding induced rotation of juxtamembrane domain and the transmembrane part likely cause the dissociation of the dimeric, intracellular domains, by which the intracellular kinase domains become accessible to their substrate tyrosine residues.

Ligand binding studies can distinguish two classes of receptors. Earlier study could determined low affinity binding sites in a form of receptor monomers, while receptor dimers possess high affinity binding properties (Boni-Schnetzler and Pilch, 1987). The presence of high affinity EGFr in monomeric as well as in dimeric forms was also described (Defize et al. 1989). Despite these discrepances the consensus exists: a/ low affinity EGFr represent a major part and high affinity EGFr account for minor part of total receptors present; b/ high affinity receptors for EGF are responsible for signalling. High-affinity binding sites account for about 18 % of total receptors, the remainder represents lower affinity binding sites (Stein et al. 2001). Contrary to those data, Moriki and coworkers (2001) presented the evidence, that EGFr dimers have conformations for both high and low affinity binding sites and that the affinities are independent of the oligomeric status of EGFr.

A similar proposal for receptor activation was previously described (Sako et al. 2000). The design suggests that dimerisation of EGFr molecules occures before the binding of a second EGF molecule, or that the two EGF molecules sequentially bind to two sites of the receptor dimer.

It is now clear that prior to ligand binding, EGF receptors are localized in specialized plasma membrane microdomains known as caveolae (Carpenter 2000). The level of EGF receptors present in caveolae is estimated to be 40-60 % of the total plasma membrane population (Mineo et al. 1999), while caveolae themselves represent a minor portion (5-10 %) of the plasma membrane. It seems likely that in the absence of a ligand caveolar EGF receptors are not a static population but rather exist in equilibrium with receptors outside these specialized regions. The addition of EGF alters the level of its receptor in caveolae and depletes the caveolar pool of EGF receptors. After EGF receptors exit caveolae they mix with the non-caveolar receptors in the plasma membrane and subsequently are trafficking to clathrin coated pits (Carpenter 2000). Following the sorting of activated EGF receptors into clathrin coated pits, the process of pit invagination and membrane fission yields intracellular coated vesicles involved in the process of receptor internalization.

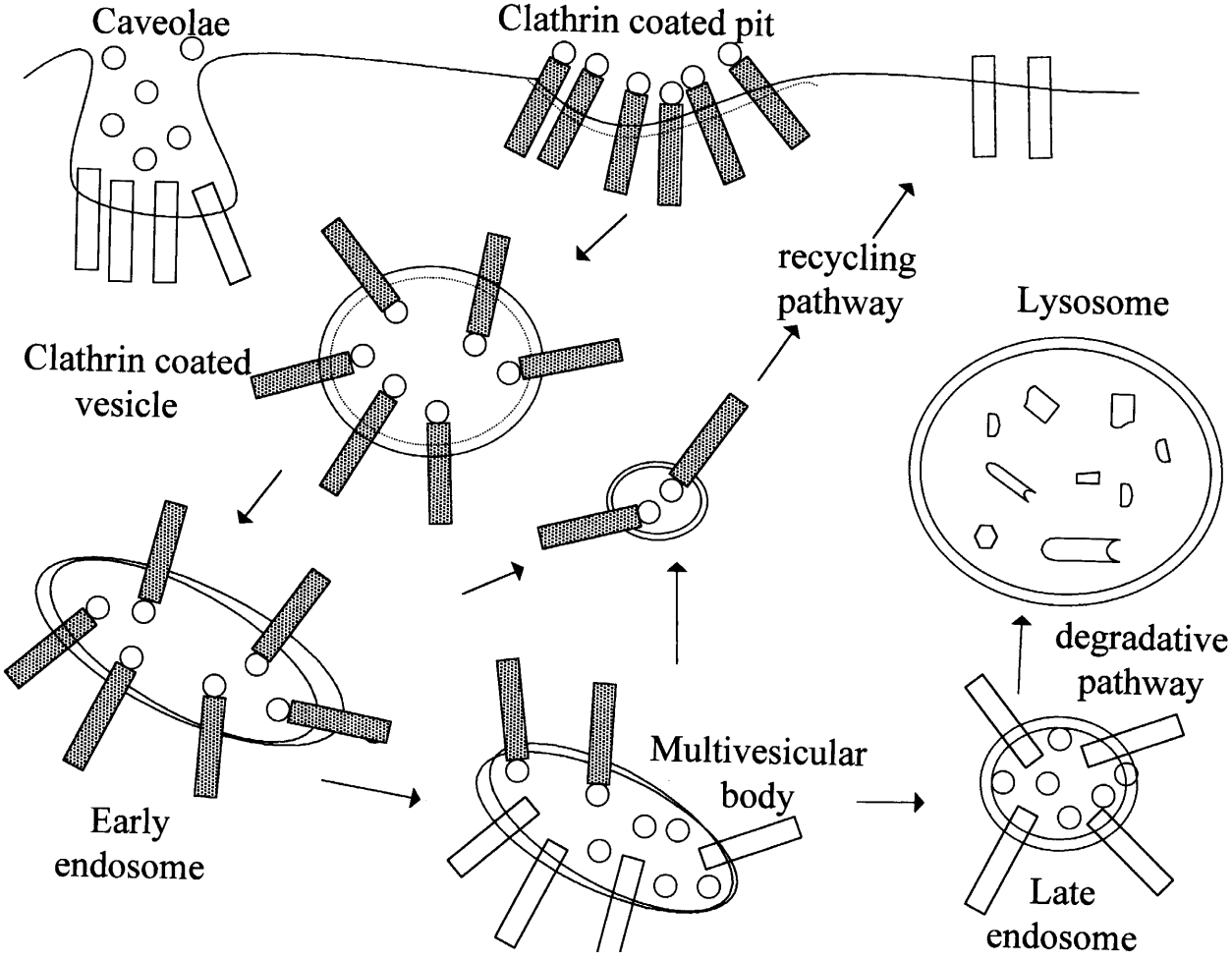

Fig. 2 Internalization of the EGF receptor. Adapted scheme of Carpenter (2000). EGFr-EGF complex migrate from caveolae to clathrin-coated pits, transformed in next step to clathrin coated vesicle. Elimination of clathrin coats produces early endosomes. This compartment then matures to form multivesicular bodies where, together with early endosomes, sorting for receptor recycling or degradation through late endosomes in lysosomes.

Transmembrane domain

Although the recent theories on receptor structure and activation are contradictory, both consider an active role of transmembrane domain (TM) in dimer EGFr formation/activation. Tanner and Kyte (1999) had determined active role of TM in such way, that interactions between TM domains provide the primary driving force for receptor dimerization. The authors propose, that unliganded extracellular domain sterically inhibits TM domain-mediated dimerization of the receptor. Activation of the receptor by EGF will occure when ligand binding induces conformational changes in extracellular domain, what will subsequent release this inhibition and permit TM-mediated EGFr homodimerization. Various experimental approaches, analytical techniques and biological materials have demonstrated that isolated TM domains from EGFr form oligomers in lipid bilayers in vitro (Jones et al. 2000) and in vivo (Mendrola et al. 2002). Thus TM domain interactions could have a role in stabilizing inactive, ligand-independent receptor dimers.

As the TM cross the lipid bilayer of the membrane, it is apparent, that the lipid environment will affect the extent of conformational changes and

spacial movement of receptor domains during the activation processes. Study with reconstituted EGFr into liposome of various lipids showed that the activity of the receptor was affected by the membrane fluidity, e.g. higher kinase activity was observed in more fluid environment. The authors (Ge et al. 2001) explain this finding by more collisions between receptor monomers what favor the formation of dimer in higher membrane fluidity. Decreased rates of EGFr dimerization at low temperature could be related to the phase transition in the membrane lipids (Moehren et al. 2002). Phosphatidylinositol and its derivatives specifically increase the affinity of EGFr for its ligand (Den Hartigh et al. 1993), while cholesterol induce aggregation of EGFr TM segment (Jones et al. 1998). It seems that activation mechanism of cell surface EGFr is influenced by a number of factors including the physical status of the environment.

Internalization

Activated EGFr rapidly internalize as the receptor-EGF complex through clathrin-coated pits by a process named ligand-induced endocytosis, into early endosomes and multivesicular bodies (Fig. 2). During passage through the endosomal compartments, these complexes are either recycled to limited extent back to cell surface or sorted to late endosomes and lysosomes, where both EGF and EGFr are proteolytically degraded. Two distinct processes have been identified that determine the fate of the internalised receptors: 1/ Participation of ubiquitin ligase (Cbl) that associates with ligand-receptor compexes in early endosomes and targets receptors for lysosomal degradation by promoting receptor ubiquitination. 2/ In the absence of Cbl, receptors are instead recycled to the plasma membrane (Yarden 2001). Internalization and subsequent degradation of EGF-occupied receptors in lysosomes is known as process of receptor down-regulation (Wiley et al. 1991). The degradation of activated receptors is much slower ($t_{1/2}$ of EGFr is ~ 2 h), than the rate of ligand internalization (only 5 min) (Burke and Wiley, 1999). These data indicate that EGF is lost three to fourfold faster than the receptor. Burke et al. (2001) explain the obtained results thus, that internalized EGFr are deactivated before degradation. Thus the loss of receptor activity is due in large part to the loss of ligand by processes as proteolysis, dissociation and endosomal sorting. The possibility of active role of internalized receptors is supported by accumulating data, that signal transduction from internalized cell surface receptors originates from endosomes. It was found (Vieira et al. 1996) that EGFr endocytosis is necessary for the activation of MAP kinase. The observed presence of intact EGFr-Shc-Grb2 complexes in the endosomes indicate that endocytosed receptors in endosomal compartment could participate in intracellular signal transduction (Sorkin 2001). This hypothesis was supported by recently described internalization of tyrosine phosphorylated EGFr associated with numerous phosphorylated proteins. These phosphoproteins differ from those associated with naturaly, surface located EGFr (Burke et al. 2001). It means that different patterns of signals arise from surface and internalized receptors. Results from the laboratory of Oksvold et al. (2001) clearly demonstrate the EGFr signalling is continuing from late endosomes. However, there are couple of counter data indicating that inhibition of EGFr internalization does not prevent activation of MAP kinase (Johannessen et al. 2000; De Graf et al. 1999). Similarly, EGFr mutant defective in internalization and undetectable in endosomes, is capable of full mitogenic and transforming activity (Carpenter 2000).

Conclusion

The signifficant role of EGFr in transducing intracellular signals engaged in various metabolic pathways, stimulation of endocrine systems, maturation and cell growth is unambiquous. Moreover engagement of EGFr in tumorigenesis make this structure the target for detailed study of receptor structure and precise molecular mechanism of its activation and intracellular signaling. The extended knowledge of the receptor structure and function will promote the desired modification of EGF receptor activity.

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References

Abe Y, Odaka M, Inagaki F, Lax I, Schlessinger J, Kohda D: Disulfide bond structure of human epidermal growth factor receptor. J Biol Chem **273**, 11150-1157, 1998

Boni-Schnetzler M**,** Pilch PF: Mechanism of epidermal growth factor receptor autophosphorylation and high-affinity binding. Proc Natl Acad Sci U S A **84**, 7832-7836, 1987

Burke PM, Wiley HS: Human mammary epithelial cells rapidly exchange empty EGFR between surface and intracellular pools. J Cell Physiol **180**, 448-460, 1999

Burke P, Schooler K, Wiley HS**:** Regulation of epidermal growth factor receptor signaling by endocytosis and intracellular trafficking. Mol Biol Cell **12**, 1897-1910, 2001

Carpenter G: The EGF receptor: a nexus for trafficking and signaling. Bioessays **22**, 697-707, 2000

Canals F: Signal transmission by epidermal growth factor receptor: Coincidence of activation and dimerization. Biochemistry **31**, 4493-4501, 1992

Defize LH, Boonstra J, Meisenhelder J, Kruijer W, Tertoolen LG, Tilly BC, Hunter T, van Bergen en Henegouwen PM, Moolenaar WH, de Laat SW**:** Signal transduction by epidermal growth factor occurs through the subclass of high affinity receptors. J Cell Biol **109**, 2495-2507, 1989

DeGraff JL, Gagnon AW, Benovic JL, Orsini MJ**:** Role of arrestins in endocytosis and signaling of alpha2-adrenergic receptor subtypes. J Biol Chem **274**, 11253-11259, 1999

Den Hartigh JC, van Bergen en Henegouwen PM, Boonstra J, Verkleij AJ**:** Cholesterol and phosphoinositides increase affinity of epidermal growth factor receptor. Biochim Biophys Acta **1148**, 249-256, 1993

Fernandes H, Cohen S, Bishayee S**:** Glycosylation-induced conformational modification positively regulates receptor-receptor association: a study with an aberrant epidermal growth factor receptor (EGFRvIII/DeltaEGF) expressed in cancer cells. J Biol Chem **276**, 5375-5383, 2001

Ge G, Wu J, Lin Q: Effect of membrane fluidity on tyrosine kinase activity of reconstituted epidermal growth factor receptor. Biochem Biophys Res Commun **282**, 511-514, 2001

Ge G, Wu J, Lin Q: Activation mechanism of solubilized epidermal growth factor receptor tyrosine kinase. Biochem Biophys Res Commun **290**, 914-920, 2002

Johannessen LE, Ringerike T, Molnes J, Madshus IH: Epidermal growth factor receptor efficiently activates mitogen-activated protein kinase in HeLa cells and Hep2 cells conditionally defective in clathrin-dependent endocytosis. Exp Cell Res **260**, 136-145, 2000

Jones DH, Barber KR, Grant CW: Sequence-related behaviour of transmembrane domains from class I receptor tyrosine kinases. Biochim Biophys Acta **1371**, 199-212, 1998

Jones DH, Ball EH, Sharpe S, Barber KR, Grant CW**:** Expression and membrane assembly of a transmembrane region from Neu. Biochemistry, **39**, 1870-1878, 2000

Lemmon MA, Bu Z, Ladbury JE, Zhou M, Pinchasi D, Lax I, Engelman DM, Schlessinger J: Two EGF molecules contribute additively to stabilization of the EGFR dimer. EMBO J **16**, 281-294, 1997

Lin SY, Makino K, Xia W, Matin A, Wen Y, Kwong KY, Bourguignon L, Hung MC: Nuclear localization of EGFR receptor and its potential new role as a transcription factor. Nat Cell Biol **3,** 802-808, 2001

Marti U, Ruchti C, Kampf J, Thomas GA, Williams ED, Peter HJ, Gerber H, Burgi U: Nuclear localization of epidermal growth factor and epidermal growth factor receptors in human thyroid tissues. Thyroid **11**, 137-145, 2001

Mendrola JM, Berger MB, King MC, Lemmon MA: The single transmembrane domains of ErbB receptors self-associate in cell membranes. J Biol Chem **277**, 4704-4712, 2002

Mineo C, Gill GN, Anderson RG**:** Regulated migration of epidermal growth factor receptor from caveolae. J Biol Chem **274**, 30636-30643, 1999

Moehren G, Markevich N, Demin O, Kiyatkin A, Goryanin I, Hoek JB, Kholodenko BN**:** Temperature dependence of the epidermal growth factor receptor signaling network can be accounted for by a kinetic model. Biochemistry **41**, 306-320, 2002

Moriki T, Maruyama H, Maruyama IN**:** Activation of preformed EGF receptor dimers by ligand-induced rotation of the transmembrane domain. J Mol Biol **311**, 1011-1026, 2001

Oksvold MP, Skarpen E, Wierod L, Paulsen RE, Huitfeldt HS: Re-localization of activated EGF receptor and its signal transducers to multivesicular compartments downstream of early endosomes in response to EGF. Eur J Cell Biol **80**, 285-294, 2001

Sako Y, Minoghchi S, Yanagida T: Single-molecule imaging of EGFR signalling on the surface of living cells. Nat Cell Biol **2**, 168-172, 2000

Sherrill JM, Kyte J: Activation of epidermal growth factor receptor by epidermal growth factor. Biochemistry **35**, 5705-5718, 1996

Sherrill JM: Self-phosphorylation of epidermal growth factor receptor is an intermolecular reaction. Biochemistry **36**, 12890-12896, 1997

Slieker LJ, Martensen TM, Lane MD: Synthesis of epidermal growth factor receptor in human A431 cells. Glycosylation-dependent acquisition of ligand binding activity occurs post-translationally in the endoplasmic reticulum. J Biol Chem **261**, 15233-15241, 1986

Sorkin A: Internalization of the epidermal growth factor receptor: role in signalling. Biochem Soc Translation **29**, part 4, 480-484, 2001

Stein RA, Wilkinson JC, Guyer CA, Staros JV: An analytical approach to the measurement of equilibrium binding constants: application to EGF binding to EGF receptors in intact cells measured by flow cytometry. Biochemistry **40**, 6142-6154, 2001

Tanner KG, Kyte J: Dimerization of the extracellular domain of the receptor for epidermal growth factor containing the membrane-spanning segment in response to treatment with epidermal growth factor. J Biol Chem **274**, 35985-35990, 1999

Vieira AV, Lamaze C, Schmid SL: Control of EGF receptor signaling by clathrin-mediated endocytosis. Science **274**, 2086-2089, 1996

Wiley HS, Herbst JJ, Walsh BJ, Lauffenburger DA, Rosenfeld MG, Gill GN: The role of tyrosine kinase activity in endocytosis, compartmentation and downregulation of the epidermal growth factor receptor. J Biol Chem **266**, 11083-11094, 1991

Wong RW, Chan SY: Epidermal growth factor receptor: a transcription factor? Trends Biochem Sci **26**, 645-646, 2001

Yarden Y: The EGFR family and its ligands in human cancer: signalling mechanisms and therapeutic opportunities. Eur J Cancer **37**, S3-S8, 2001

Zhou M, Felder S, Rubinstein M, Hurwitz DR, Ullrich A, Lax I, Schlessinger J: Real-time measurement of kinetic of EGF binding to soluble EGF receptor monomers and dimers support the dimerization model for receptor activation. Biochemistry **32**, 8193-8198, 1993

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