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Role of Nuclear EGFR During Cellular Radiation Response

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Emerging evidence suggests the existence of a new mode of epidermal growth factor receptor (EGFR) signalling in which activated EGFR undergoes nuclear translocation. We provide evidence that the nuclear EGFR transport is a stress specific cellular reaction, which is linked to src dependent EGFR internalization into caveolae. Internalized EGFR is sorted into a peri-nuclear localization. This peri-nuclear EGFR may serve as a reservoir for nuclear transport which is regulated by PKC ε. Nuclear EGFR induces transcription of genes essential for cell proliferation and cell cycle regulation. In addition, nuclear EGFR has physical contact with compounds of the DNA repair machinery and is involved in removal of DNA-damage. The exact role of nuclear EGFR has to be elucidated in future experiments.

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The outstanding role of EGFR

A high proportion of human tumor cells is characterized by overexpression of epidermal growth factor receptor (EGFR), a protein that promotes resistance to chemo- and radiotherapy.[1-5] EGFR protein can be activated through phosphorylation at specific amino acid residues in response to ligand binding (EGF, TGF alpha and Amphiregulin) as well as after exposure to a variety of unspecific stimuli like ionizing radiation, UV-radiation, hypoxia, hyperthermia, oxidative stress and trans-activation by G-protein coupled receptors.[6-13] Both ligand-dependent as well as ligand-independent phosphorylations of EGFR result in receptor internalization and intracellular signalling.[4,5,14-17] Up to date internalization is assumed to be essential for receptor silencing and inactivation. Indeed, EGF treatment results in internalization of EGFR into coated pits followed by receptor degradation.[18] Exposure to oxidative stress can lead to internalization of EGFR into caveolae, however this process is associated with peri-nuclear accumulation of EGFR and persistent kinase activity, as reported by Khan.[12] The broad inducibility of EGFR activation and internalization by cellular stress, suggest an essential role of EGFR during regulation of cellular survival. The special role of nuclear EGFR has been underlined by the clinical observations, that detection of nuclear EGFR in tumors biopsies is strongly correlated with treatment resistance and bad prognosis.[1,19-22]

Radiation induced internalization of EGFR

A characteristic compound of caveolae is the protein caveolin. Caveolin gene family consists of three members: CAV1, CAV2, and CAV3, coding for the proteins caveolin-1, caveolin-2 and caveolin-3, respectively. Caveolins associate with cholesterol and sphingolipids in specific areas of the cell membrane to form caveolae. Caveolae are involved in receptor independent endocytosis and intracellular signalling.[23] In addition, caveolin-1 is a transmembrane protein and an essential component during interactions of integrin receptors with cytoskeleton-associated molecules.[24] Caveolae contain a high variety of proteins essential for signalling. Caveolae and associated proteins form the so called caveosome which can fuse with the early endosomes.[25] Moreover, caveolin-1 is found at many intracellular locations. Variations in subcellular localization are paralleled by a plethora of ascribed functions for this protein. These observations suggest a general function of caveolae as an intracellular signalling platform. In agreement with that, compartmentation into caveolae prevents EGFR degradation and simultaneously enables intracellular EGFR kinase linked signalling.[12] These findings suggest a new function of EGFR – depending on its intracellular localization –, which supplements its functions described so far. Ionizing radiation results in fast src kinase stabilization, activation and subsequent src mediated caveolin-1 Y14- and EGFR Y845-phosphorylations. Both phosphorylations are radiation specific and can not be observed after

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treatment with EGF, which suggests caveolae-sorting of EGFR as a stress-associated event.[26] Inhibition of EGFR by the antibody Erbitux results in a strong accumulation of caveolin/EGFR complexes within the cytoplasm, which can not be further increased by irradiation. Radiation-induced caveolin-1- and EGFR-phosphorylations are associated with nuclear EGFR transport.[26,27] As shown by the specific inhibitor PP2, blockage of src activity inhibits caveolin-1-phosphorylation and decreased nuclear transport of EGFR.[26]

Translocation of EGFR from caveolae into endoplasmatic reticulum (ER)

Nuclear localization of the EGF receptor requires endocytosis and association of the receptor with the karyopherin carrier nuclear import system.[27] However, this association does not explain how a transmembrane receptor is processed into a nuclear non-membrane-bound receptor. As cells do have protein complexes that translocate proteins into and out of lipid bilayers, Liao et al. explored the possibility, that the Sec61 translocon could mediate nuclear transport of the EGF receptor.[27-29] EGFR located within the membrane of late endosomes is transferred to the membranes of Golgi apparatus by membrane fusion and at least locates in the ER membrane. For nuclear transport EGFR has to be set free from ER-membrane to become a cytosolic protein and to admit access of the karyopherin system to the intrinsic nuclear localization site of the EGFR. The Sec61 translocon is located exclusively in the endoplasmic reticulum (ER) and ER/Golgi transitional region and functions to insert secretory and transmembrane proteins into the ER during protein synthesis.[30,31] The translocon is bidirectional and retrotranslocates also proteins from ER membrane to the cytosol.

EGFR transport into nucleus

Passage through the nuclear pore complex (NPC) needs binding to nuclear transport receptors. Many proteins are imported via karyopherin-ß (often using karyopherin-α as an adaptor). Indeed it was shown, that after irradiation the EGFR is found in complex with karyopherin-α and RAN-GTP.[5] Prerequisite for karyopherin-binding is the presence of a nuclear localization site (NLS) within the cargo protein. Classic NLSs contain one or two clusters of basic residues. Monopartite NLSs, exemplified by the SV40 large-T antigen, have a single cluster of 4–5 basic residues, whereas bipartite NLSs, such as that of nucleoplasmin, have a second basic cluster located ~10–12 residues downstream of the first cluster.[32] Molecular recognition of NLSs is essential for the formation of the import complex. Lin et al.[33] reported identification of a putative NLS within the EGFR sequence and proved the function. Interestingly, we observed phoshorylation of EGFR at residue T654, which is located within this putative EGFR NLS, after radiation-induced nuclear EGFR transport. Furthermore, we identified PKC ε as the kinase responsible for this modification.[34] So far it is not resolved whether this phosphorylation regulates assembly of the EGFR-karyopherin α/ß transport complex within the cytosol, or disassembly within nucleus. However, first results with cells expressing EGFR mutated in NLS (Dittmann et al., unpublished data) argue for a regulative role of T654 phosphorylation during assembly of transport complex after radiation. Nuclear EGFR accumulation results from a balance of import and export processes.[5] Recent evidence suggest, that nuclear export of EGFR may involve exportin CRM1.[35] Existence of nuclear export sequences within EGFR sequence however, has not been demonstrated.

Function of nuclear EGFR

Nuclear EGFR detection was first reported in hepatocytes that underwent regeneration and in primary adrenocortical carcinomas.[36,37] High levels of EGFR were detected in the nuclei of many tumours, including those of adrenocorticord, breast, bladder, skin, thyroid and oral cavity.[37,38,39,36,40,22] Nuclear EGFR appears to be the full-length phosphorylated receptor.[5,33,41] Nuclear EGFR positively correlates with Ki-67, expression, an indicator of proliferation.[40] Consequently, a function of nuclear EGFR as transcriptional activator was suggested. Indeed, transactivation domains within EGFR, HER-2 and HER-4 were identified and found to be functional.[33,42] Nuclear EGFR and HER-2 were shown to associate with specific DNA sequences designated AT-rich sequence and HER-2-associated sequence, respectively.[33,42] Promoters that are targeted by nuclear EGFR are those of cyclin D1, iNOS and B-Myb.[33,43,44] Given the notion that ErbB receptors lack a putative DNA-binding domain, it is suspected that these receptors first associate with DNA-binding transcription factors and then enhance target gene transcription via their intrinsic transactivational activity. In this regard, nuclear EGFR interacts with STAT3 and co-regulates iNOS expression.[44] Furthermore cooperation of nuclear EGFR with the transcription factor E2F1 activates expression of B-Myb, a positive regulator of G1/S cell cycle progression.[43] The observation that nuclear EGFR is phosphorylated at autophosphorylation sites indicates that kinase activity of EGFR is present within nucleus and suggests that this kinase activity may be relevant for the function of nuclear EGFR. Indeed Wang et al. could demonstrate that, PCNA is subject to tyrosine phosphorylation at a specific site in an EGFR dependent manner and that this phosphorylation enhances PCNA stability on the chromatin.[45] Thus these data link tyrosine kinase activity of nuclear EGFR with cell proliferation and DNA repair by regulating PCNA function.

In addition, Bandyopadhyay et al. described that nuclear EGFR can interact with DNA-repair and cell survival directly.[46] They described physical interaction of EGFR with DNA dependent kinase (DNA-PK). Furthermore they demonstrated that blocking EGFR signalling by Erbitux, an anti-EGFR monoclonal antibody, resulted in reduction of nuclear DNA-PK protein and kinase activity, implicating a role of EGFR in regulation of DNA repair. Indeed it could be shown that nuclear EGFR is associated with phosphorylation of DNA-PK at residue T2609, which stands for DNA-PK activity

during non-homologous end-joining DNA-repair.[5] Blockage of nuclear EGFR transport by Erbitux decreased DNA-PK activity and consequently increased residual DNA-damage and reduced survival after radiation treatment.[47] These observations suggest a crucial role of nuclear EGFR for regulation of DNA-repair following treatment with genotoxic substances.

Translational Approach

Earlier we could show that either the Bowman Birk proteinase inhibitor BBI or its derivate P-Tyr can act as a selective radioprotectors in normal or tumor cells characterized by a wild type TP53, when cells are treated prior to irradiation.[48-50] It was shown, that for BBI-mediated radioprotection the activation of DNA-PK activity is essential. Consequently, DNA-repair quality was improved, measured as reduction of residual dicentric chromosomes.[51] These data suggest a clear effect of BBI and P-Tyr upon DNA-repair in general and presumably on DNA-double strand break repair especially. However, the molecular mode of action was unknown. Recent results form our laboratory indicate, that BBI and P-Tyr interfere with EGFR phosphorylation and radiation-induced EGFR transport into the cell nucleus.[52,53] This transport is involved in regulation of DNA-PK activity which is essential for DNA-repair.[5,54] Thus when given before irradiation, BBI and P-Tyr both stimulate EGFR nuclear transport and consequently improve radiation-induced DNA-damage and enhance cellular survival.

Summary

Current knowledge about nuclear transport is summarized in Figure 1. Nuclear localization of EGFR was observed either after cell stimulation with EGF or after treatment with genotoxic substances. However, the scenario described herein in fact is oversimplified, since the effects of nuclear EGFR are superimposed by the cytosolic signalling of membrane associated EGFR. In addition, nuclear EGFR interacts with other members of the erbB-receptor family also detected within the nucleus. Nevertheless the relevance of nuclear EGFR for cell survival and DNA-repair is beyond doubt and these data may not only improve our knowledge on basic mechanisms of radiation sensitivity / resistance, but also will promote new strategies for clinically molecular targeting.

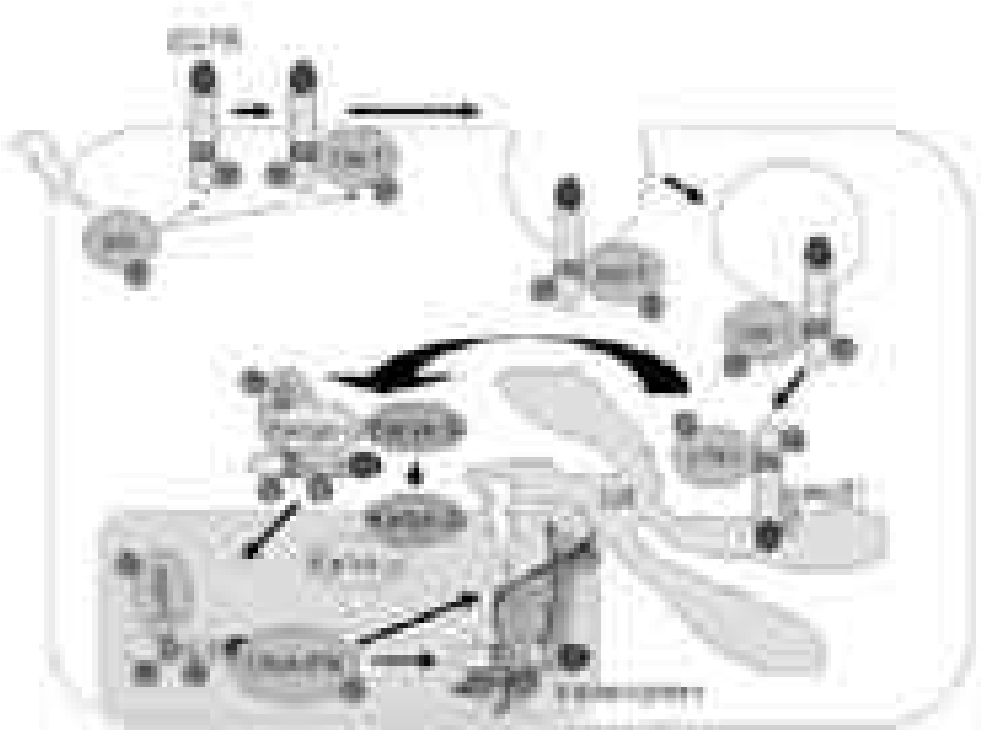

Figure 1. Role of nuclear EGFR during cellular radiation response
Radiation activates src kinase in a so far not understood manner. Src phosphorylates EGFR at residue residue Y845 and caveolin1 at residue Y14, which seems to be signal for complex formation and internalization into caveolae. EGFR containing caveolae are transported to the Golgi / endoplasmatic reticulum (ER) in a microtubule dependent way and fuse with ER membrane. EGFR is found in complex with translocon Sec61 and set free by its action into the cytosol. EGFR is phosphorylated at residue T654 by means of PKCε, which induces binding of karyopherin α and binding of karyopherin ß. This process enables transport through nuclear pore into nucleus. Karyopherins dissociate from nuclear complex and are exported to cytosol back. Nuclear EGFR either interacts with DNA-PK and is involved in activation of kinase activity essential for non-homologous end-joining DNA-repair, or acts as a transcription factor regulating expression of essential genes.

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