

Commentary

Cortactin overexpression results in sustained epidermal growth factor receptor signaling by preventing ligand-induced receptor degradation in human carcinoma cellsAgnes GSH van Rossum¹, Johan Gibcus², Jacqueline van der Wal² and Ed Schuuring²¹Department of Pathology, Leiden University Medical Center, Leiden, The Netherlands²Department of Pathology, University Medical Center Groningen, The NetherlandsCorresponding author: Ed Schuuring, e.schuuring@path.umcg.nl

Published: 25 August 2005

This article is online at <http://breast-cancer-research.com/content/7/6/235>

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Breast Cancer Research 2005, **7**:235-237 (DOI 10.1186/bcr1316)**Abstract**

The chromosome 11q13 region is frequently amplified in human carcinomas and results in an increased expression of various genes including cortactin, and is also associated with an increased invasive potential. Cortactin acts as an important regulator of the actin cytoskeleton. It is therefore very tempting to speculate that cortactin is the crucial gene within the 11q13 amplicon that mediates the invasive potential of these carcinomas. Cortactin also participates in receptor-mediated endocytosis, and recent findings have shown that, during receptor internalization, cortactin overexpression inhibits the ubiquitylation-mediated degradation of the epidermal growth factor receptor, resulting in a sustained ligand-induced epidermal growth factor receptor activity.

Cell structure is maintained by the combined action of microtubules, intermediate filaments and the actin cytoskeleton. Actin filaments contribute to alterations in the cell shape, motility, adhesion, polarization, contraction, cytokinesis, signal transduction, endocytosis and intracellular vesicle trafficking. Cortactin [1], as a regulator of actin cytoskeleton organization, is involved in many of these processes (reviewed in [2,3]). For instance, many observations revealed that cells overexpressing cortactin show enhanced cell migration, invasion and increased metastatic potential *in vivo* [4-6]. Furthermore, downregulation of cortactin in highly invasive cells *in vitro* using small RNA interference (van Rossum, Moolenaar, Schuuring, unpublished research), deletion mutants or microinjection of antibodies resulted in a decreased invasive potential [4,6,7].

Interestingly, human cortactin was identified as one of the overexpressed genes within the amplified chromosome 11q13 region [8,9]. The 11q13 region is frequently amplified in human malignancies such as breast carcinomas (13%) and head/neck carcinomas (36%) [10,11]. In these patients, both 11q13 amplification and cortactin overexpression correlate

with markers indicative of poor prognosis, such as the presence of lymph node metastases [10-12]. Because cortactin acts as a regulator of the actin cytoskeleton integrity, it is tempting to speculate that cortactin mediates the invasive and metastatic potential of carcinomas with 11q13 amplification.

With the identification of the growing number of cortactin-interacting proteins, novel functions of cortactin have emerged [2,13]. Cortactin has been implicated in endocytosis by its localization in endosomal vesicles [14]. Furthermore, GTPase dynamin-2 was identified as a cortactin-interacting protein and, as such, linked the actin cytoskeleton to clathrin-dependent endocytosis [15]. More recent studies [16-18] provide convincing evidence that cortactin is involved in dynamin-mediated, clathrin-dependent endocytosis. Many growth factor receptors, including epidermal growth factor receptor (EGFR), are regulated via this classic endocytic pathway (reviewed in [19,20]). The ligand-induced EGFR is trapped on the plasma membrane into clathrin-coated pits, which is followed by the formation of clathrin-coated vesicles by dynamin-mediated fission and fusion. These vesicles form early endosomes that can mature towards late lysosomal endosomes. Under certain circumstances, however, the EGFR might be prevented from entering the lysosomal endosome and may become recycled to the cell surface, a mechanism that fine-tunes the activity of the EGFR [19].

It was recently demonstrated that ubiquitylation by Cbl triggers the EGFR to enter lysosomal endosomes, resulting in its degradation (reviewed by [20]). For instance, the EGFR with mutations at the Cbl-docking site will not be degraded and is recycled back to the plasma membrane. Other members of the ErbB-family, such as ErbB2, that lack a docking site for Cbl, can form heterodimers with the EGFR.

This thereby reduces the ability of the EGFR to associate with Cbl, resulting in an increased recycling of the receptor. Finally, EGFRvIII, a mutant found in glioblastoma that lacks a portion of the extracellular domain, showed defects in internalization and ubiquitylation by Cbl.

Timpson and colleagues hypothesized in an earlier issue of *Cancer Research* that cortactin is also involved in modulating EGFR activity [21]. They showed that the EGFR is degraded less efficiently in cells with increased cortactin expression, and that the EGFR activity upon ligand stimulation is sustained. In agreement with these experiments, expression of cortactin correlated well with ligand-stimulated EGFR signal transduction in cell lines derived from head/neck carcinomas. In addition, increased expression levels of cortactin did not significantly influence EGFR internalization, but resulted in accelerated recycling – leading the authors to speculate that cortactin might have an effect on the transfer of the EGFR from the early to the late lysosomal endosomes. Indeed, the EGFR was not efficiently ubiquitylated in cells that overexpressed cortactin, resulting in inhibition of the ligand-induced down-regulation of the receptor and sustained epidermal growth factor-induced Erk activity. The decrease in ubiquitylation was accompanied by the inhibition of Cbl binding to the EGFR.

The mechanism for the observed effects of cortactin overexpression on Cbl function is presently unclear. The authors discuss several possibilities to explain the impaired Cbl activity [21]. Activated Cdc42 binds to Cbl via p85Cool-1/ β -Pix and prevents Cbl interacting with the EGFR, thus preventing receptor ubiquitylation [22]. Fgd1, a Cdc42 guanine nucleotide exchange factor, was recently identified to interact with cortactin [23]. Overexpression of cortactin might therefore activate Cdc42 via Fgd1, leading to Cdc42/p85Cool-1/ β -Pix/Cbl complex formation and consequently reducing the interaction of Cbl to the EGFR. Timpson and colleagues [21] demonstrated that Cbl tyrosine phosphorylation as well its interaction with the EGFR was reduced in cortactin-overexpressing cells, whereas the amount of Cbl was not affected. Cbl is a substrate for nonreceptor tyrosine kinase Src, and phosphorylation of Cbl is necessary to activate its ubiquitin ligase activity [24]. Overexpression of cortactin, one of the most prominent substrates for Src, might therefore sequester Src from Cbl to prevent Cbl phosphorylation and thereby prevent its ability to ubiquitylate the EGFR. Finally, cortactin overexpression did not cause EGFR internalization, but after 60–120 min of epidermal growth factor stimulation the EGFR reappeared on the cell surface. At the same time, degradation of the EGFR was also observed in cortactin-overexpressing cells. Since no degradation of the EGFR was observed until 30 min of epidermal growth factor stimulation in these cells, the enhancement of Erk activation after 5–10 min of epidermal growth factor stimulation suggests an altered trafficking between specific endosomal compartments [21]. However, it is not known exactly how cortactin affects EGFR trafficking.

In conclusion, it is presently unclear how cortactin overexpression impairs Cbl phosphorylation and consequent ubiquitylation of the EGFR. Recent reports provide convincing evidence that cortactin is an important regulator during receptor-mediated endocytosis by its interaction with dynamin and actin [16–18]. However, the work by Timpson and colleagues [21] demonstrated that cortactin can interfere directly or indirectly with ligand-induced downregulation of the EGFR. These authors showed that increased expression of cortactin resulted in the inhibition of EGFR degradation and sustained EGFR activity. Together with the observation that the EGFR has already been reported to be activated in carcinomas of the head/neck region due to EGFR DNA amplification [25], these observations imply that constitutive EGFR activity is present in a significant number of these carcinomas. It would be of interest to learn about the role of cortactin expression in regulating the endocytosis of other receptors and cell adhesion molecules.

Competing interests

The author(s) declare that they have no competing interests.

Acknowledgements

AGSHvR was supported by grant NKB-RUL 98-1647 of the Dutch Cancer Society and JG by the Research School GUIDE. The authors thank Jeroen Guikema for critical reading of the manuscript.

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