Experimental Cell Research 356 (2017) 93–103

Contents lists available at ScienceDirect

Experimental Cell Research

journal homepage: www.elsevier.com/locate/yexcr

EGFR-mediated apoptosis via STAT3

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ARTICLE INFO

Keywords:
Epidermal Growth Factor Receptor
STAT3
Apoptosis
MDA-MB-468 Cells
A431 Cells
Oncostatin M
Stattic
S3I-201

ABSTRACT

The Epidermal Growth Factor Receptor (EGFR) is a cell surface receptor with primary implications in cell growth in both normal and malignant tissue. Paradoxically, cell lines that hyperexpress the EGFR have been documented to undergo receptor-mediated apoptosis. The underlying mechanism by which EGF-induced apoptosis occurs however remains inexplicit. In an attempt to identify this mechanism, we assessed downstream effectors of EGFR in MDA-MB-468 cells during conditions of EGF-induced apoptosis. The effector assessment revealed STAT3 as a potential mediator of EGF-induced apoptosis. Alternative strategies for activating STAT3, independent of EGFR stimulation, resulted in the induction of the apoptotic pathways. A reduction in STAT3 expression via RNAi resulted in a significant attenuation of EGF-induced PARP cleavage. Our findings support STAT3 as a positive mediator of EGF-induced apoptosis in MDA-MB-468 cells.

1. Introduction

The Epidermal Growth Factor Receptor (EGFR) is a transmembrane receptor tyrosine kinase that plays critical roles in cell growth, tissue development, and overall cellular homeostasis [1,2]. Despite these critical physiological roles, there are a myriad of human malignancies [3–5] that are characterized by hyper-activated EGFR signaling. This is due to either receptor overexpression or activating mutations of the receptor [4,6]. The FDA has approved pharmacological agents that antagonize ligand binding and inhibit EGFR kinase activity for the treatment of patients with cancers characterized by hyper-activated EGFR signaling [7–9]; however, these drugs have off-target effects (i.e. colitis, corneal erosions and dermatitis) and cancers often become desensitized to these agents over time [10–12]. Thus, there remains a need to develop drugs that more aggressively and specifically target those cancers with enhanced EGFR signaling, but allow normal, healthy cells to perform their biological roles.

One potential strategy for doing this is to hijack intrinsic signaling pathways and exploit them to compromise the cell's health. Cell lines with EGFR overexpression either naturally (A431 and MDA-MB-468 cells [13,14]) or via bioengineering (Rat-1 fibroblasts) [15], undergo EGF-induced apoptosis [4,16,17]. Understanding the molecular mechanism by which this occurs will identify new potential pharmacological targets that can induce death in cells that overexpress the EGFR (i.e. cancer cells).

Through an effector screen, Signal Transducer and Activator of Transcription 3 (STAT3) was identified as a plausible mediator of EGFR-induced apoptosis. STAT3 is one of seven members of the STAT family of transcription factors, namely, STAT1, STAT2, STAT3, STAT4, STAT5a, STAT5b, and STAT7. STAT activation is critical for a number of biological processes, including cell proliferation, survival, differentiation, development and inflammation [18]. STAT3 is a DNA-binding transcription factor known to mediate normal cellular processes upon activation by growth factors, ligands and cytoplasmic cytokines, such as cytokine receptor-associated Janus kinases (JAKs) [19–22]. These upstream protein components phosphorylate STAT3 at its unique, critical tyrosine residues, primarily Tyr705 [19,20]. The EGFR has also been reported to directly tyrosine phosphorylate STAT3 [23,24]. STAT phosphorylation occurs at the cytoplasm, which induces STAT homo- and heterodimer formation between two monomers via their Src homology 2 (SH2) domain interactions. From the cytoplasm, activated STAT dimers translocate and accumulate in the nucleus, where they initiate and mediate gene transcription by binding to DNA response elements [19]. This results in either upregulation or downregulation of the biological effectors and subsequent cellular processes that are critical for cellular homeostasis.

STAT3 has been implicated in the post-transcriptional modification of cellular processes, positively and negatively affecting cell growth and proliferation. Aberrant STAT3 signaling and constitutive activity has been reported in a number of cancers [18,25]. Elevated basal STAT3 activity has been reported in 30–60% of primary mammary malignancies, with reports of it being required for tumor cell progression and metastasis [23]. However, prior to being implicated in cancer, STAT3-mediated programmed cellular death was found to be associated with

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http://dx.doi.org/10.1016/j.yexcr.2017.04.016
Received 18 February 2017; Received in revised form 6 April 2017; Accepted 8 April 2017
Available online 19 April 2017

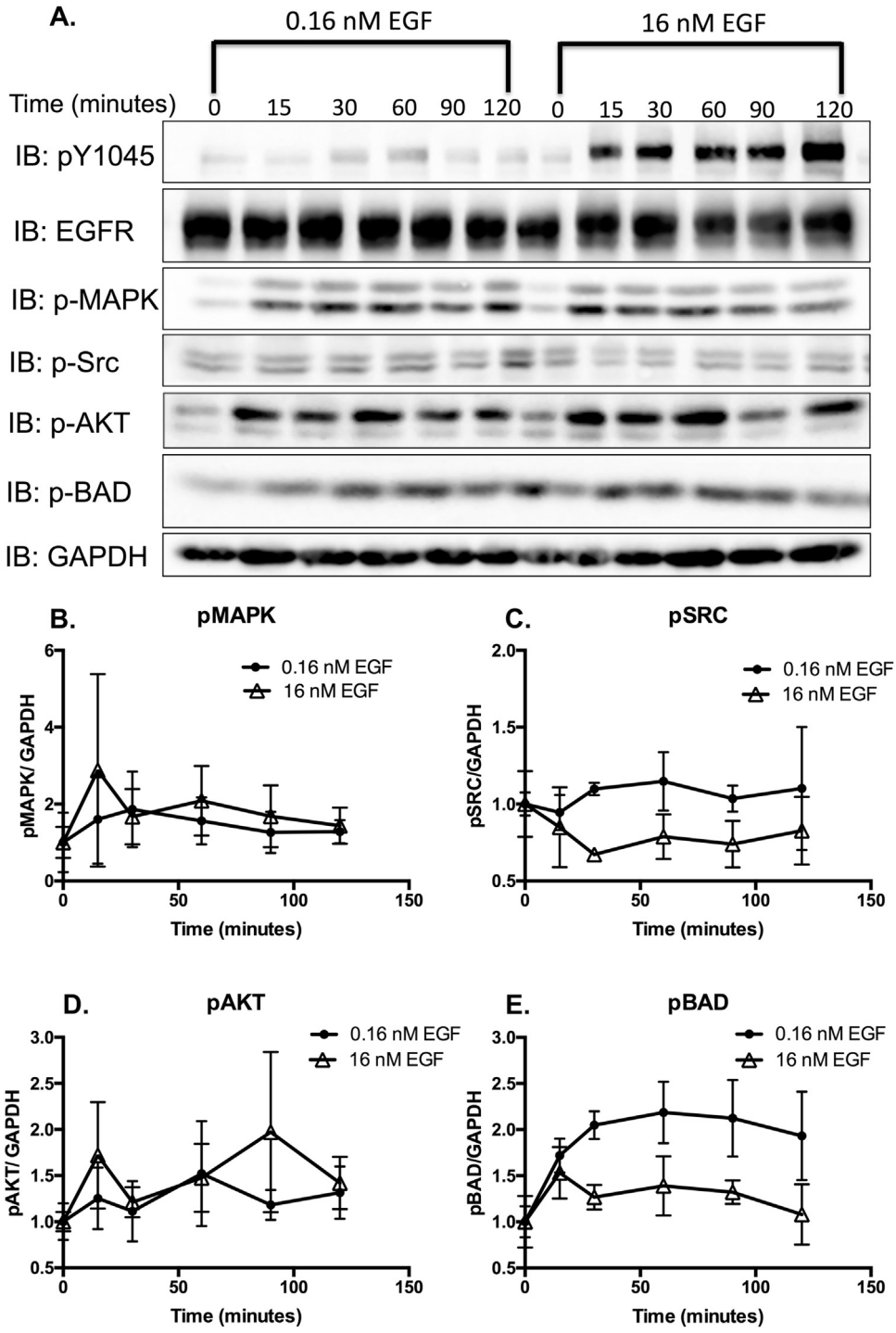

Fig. 1. Phosphorylation of MAPK, SRC, AKT, and BAD in response to high and low EGF concentrations. **A.** Serum-starved MDA-MB-468 cells were stimulated with low (0.16 nM) or high (16 nM) EGF ligand for 0–120 min. Cell lysates (20 μg) were resolved by 12% SDS-PAGE and were assessed for the indicated proteins via immunoblot analysis. Band intensities from the immunoblot data of pMAPK (**B.**), pSRC (**C.**), pAKT (**D.**) and pBAD (**E.**) were quantified, normalized to GAPDH levels, and plotted as the relative level compared to cells with no treatment. Data are expressed as the average ± Standard Error of the Mean (SEM; n=3).

and required for mammary gland involution [26,27]. Additionally, STAT3 has been previously identified as a key mediator of apoptosis in murine pro-B cells [28], myeloid leukemia [29], and prostate cancer [30]. These conflicting roles for STAT3 in the context of normal and malignant cellular biology highlight the need for a better understanding of this protein in EGFR signaling mechanisms.

The overarching goal of this study was to identify intermediates in EGFR-mediated apoptosis. STAT3 was identified as being specifically activated under apoptosis-inducing conditions. Utilizing siRNA targeting STAT3 resulted in a significant attenuation of EGF-induced apoptosis. Additionally, EGFR-independent activation of STAT3 through cytokine stimulation promoted apoptosis, as measured by PARP and Caspase-3 cleavage. From these findings, we conclude that STAT3 is required for EGFR-mediated cell death via PARP cleavage and subsequent activation.