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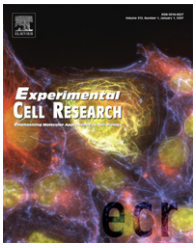

Research Article

Characterization of E-cadherin-dependent and -independent events in a new model of c-Fos-mediated epithelial–mesenchymal transition

Jakob Mejlvang[a,b], *Marina Kriajevska*[a], *Fedor Berditchevski*[c], *Igor Bronstein*[d], *Eugene M. Lukanidin*[b], *J. Howard Pringle*[e], *J. Kilian Mellon*[a], *Eugene M. Tulchinsky*[a,*]

[a]*Department of Cancer Studies and Molecular Medicine, University of Leicester, Hodgkin Bldg., Lancaster Rd, LE1 9HN, Leicester, UK*
[b]*Department of Molecular Cancer Biology, Danish Cancer Society, Copenhagen, Denmark*
[c]*Cancer Research UK Institute for Cancer Studies, University of Birmingham, Birmingham, UK*
[d]*Medical Research Council National Institute for Medical Research, The Ridgeway, Mill Hill, London, UK*
[e]*Department of Cancer Studies and Molecular Medicine, Robert Kirkpatrick Clinical Sciences Bldg., Leicester, UK*

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ABSTRACT

Fos proteins have been implicated in control of tumorigenesis-related genetic programs including invasion, angiogenesis, cell proliferation and apoptosis. In this study, we demonstrate that c-Fos is able to induce mesenchymal transition in murine tumorigenic epithelial cell lines. Expression of c-Fos in MT1TC1 cells led to prominent alterations in cell morphology, increased expression of mesenchymal markers, vimentin and S100A4, DNA methylation-dependent down-regulation of E-cadherin and abrogation of cell–cell adhesion. In addition, c-Fos induced a strong β-catenin-independent proliferative response in MT1TC1 cells and stimulated cell motility, invasion and adhesion to different extracellular matrix proteins. To explore whether loss of E-cadherin plays a role in c-Fos-mediated mesenchymal transition, we expressed wild-type E-cadherin and two different E-cadherin mutants in MT1TC1/c-*fos* cells. Expression of wild-type E-cadherin restored epithelioid morphology and enhanced cellular levels of catenins. However, exogenous E-cadherin did not influence expression of c-Fos-dependent genes, only partly suppressed growth of MT1TC1/c-*fos* cells and produced no effect on c-Fos-stimulated cell motility and invasion in matrigel. On the other hand, re-expression of E-cadherin specifically negated c-Fos-induced adhesion to collagen type I, but not to laminin or fibronectin. Of interest, mutant E-cadherin which lacks the ability to form functional adhesive complexes had an opposite, potentiating effect on cell adhesion to collagen I. These data suggest that cell adhesion to collagen I is regulated by the functional state of E-cadherin. Overall, our data demonstrate that, with the exception of adhesion to collagen I, c-Fos is dominant over E-cadherin in relation to the aspects of mesenchymal transition assayed in this study.

* *Corresponding author*. Fax: +44 252 116 2525616.
E-mail address: et32@le.ac.uk (E.M. Tulchinsky).

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Introduction

AP-1 transcription factors (Jun/Jun homodimers and Fos/Jun heterodimers) are activated on transcriptional and post-translational levels in response to a multitude of extracellular stimuli. Activated AP-1 binds TREs (TPA-responsive elements) located in enhancers of target genes to up-regulate transcription by recruiting transcriptional co-activators CBP/p300 or JAB1 [1]. AP-1 has been implicated in most fundamental biological processes including cell proliferation [2,3], differentiation [4–6], apoptosis [7–9] and tumorigenesis [9,10]. The prototypical member of the Fos protein family, the transcription factor c-Fos efficiently transforms rodent fibroblasts *in vitro* [11,12], induces formation of osteosarcomas in transgenic animals [13,14] and is required for Src- and Ras-induced oncogenic transformation [10]. The transforming ability of c-Fos and the fact that its expression is tightly linked to mitogenic stimulation by growth factors suggest a role for c-Fos in the control of cell growth [3]. Although c-*fos* knockout mice are growth retarded, c-*fos*–/– fibroblasts proliferate normally, likely due to the fact that other Fos family members (FosB, Fra-1 or Fra-2) compensate for the lack of c-Fos [15,16]. The particular function of Fos proteins in tumor cells is context-specific, and the expression of v-Fos in primary or immortalized human fibroblasts does not alter cell proliferation [17], whereas in epithelial hepatocytes, c-Fos-estrogen receptor (Fos-ER) chimera inhibits cell growth [8]. Clearly, the function of c-Fos in tumorigenesis is not restricted to cell cycle control. Since c-Fos, v-Fos and Fra-1 proteins activate the expression of genes implicated in invasion and angiogenesis and influence cell motility [18–23], a role for Fos proteins at later stages of epithelial tumorigenesis has been proposed. The essential role of c-Fos in progression from non-invasive papilloma to malignant tumors has been directly shown in the multistep skin carcinogenesis model using c-*fos* null mice [24]. Relevant to these data, Fos-ER induces epithelial–mesenchymal transition (EMT) in mouse non-tumorigenic Ep-1 cells [25].

EMT is a regulated phenotypic modulation of epithelial cells, which results in the generation of invasive, motile cell phenotypes. EMT occurs in embryogenesis during gastrulation and neural crest cell migration and at the later stages of epithelial tumorigenesis leading to the formation of metastatic tumors [26]. A hallmark of EMT is the dissociation of adherens junctions, the homophilic E-cadherin-mediated epithelial cell–cell adhesion contacts. Loss of E-cadherin function during embryonic development and tumor progression is believed to have implications for cellular signaling networks [27,28]. Disappearance of E-cadherin may affect signaling by influencing activity of Rho proteins [29], via modulation of receptor tyrosine kinases (EGFR, ErbB2, IGFR or EPHA2) function [30–32] or by activating the β-catenin pathway [33–35]. β-catenin interacts with the C-terminal domain of E-cadherin and links the E-cadherin complexes to the actin cytoskeleton providing stable cell adhesion. A small pool of free β-catenin may interact with TCF/LEF transcription factors and activate transcription by providing a transactivation domain [36]. β-catenin signaling contributes to tumorigenesis by transcriptional activation of genes regulating cell cycle progression and tumor cell invasion. The signaling pool of β-catenin may be sequestered by E-cadherin leading to the inhibition of β-catenin signaling. In the last 5 years, progress has been made in understanding mechanisms responsible for the silencing of E-cadherin in tumor progression. Transcriptional repressors belonging to three protein families, Snail/Slug, ZEB-1(DeltaEF1)/ZEB-2(SIP1) and E12/E47 have been shown to directly interact with *e-cadherin* promoter DNA and actively repress transcription. Other mechanisms of functional inhibition of E-cadherin include gene mutations [37–39] and hypermethylation of a CpG island near the *e-cadherin* transcription start site [40,41]. Loss of E-cadherin expression or mutations in the gene are associated with several forms of epithelial cancer [42], and an invasion suppressor role for E-cadherin has been demonstrated in a transgenic mouse model [43]. The EMT of Ep-1 cells induced by the activation of Fos-ER was accompanied by changes in gene expression program involving down-regulation of E-cadherin and up-regulation of mesenchymal markers and several extracellular matrix-degrading proteases [25]. Loss of E-cadherin resulted in nuclear re-localization of β-catenin and β-catenin/LEF-dependent transcription [44]. Activation of this pathway was shown to be essential for the proliferation and survival of Ep-1 cells undergoing Fos-ER-mediated EMT [34].

Thus, the considerable amount of experimental data suggests a regulatory role for c-Fos at later stages of epithelial tumorigenesis. In addition, deregulation of c-Fos expression has been reported in several forms of human cancer (for references, see [45]). However, c-Fos function in established carcinoma cell cultures has not been studied. In this study, we show that epithelioid mouse mammary adenocarcinoma cells undergo EMT in response to c-Fos. Morphological transition of these cells was concomitant with the down-regulation of E-cadherin. We address the mechanisms of E-cadherin down-regulation and its involvement in c-Fos-mediated EMT in carcinoma cells.

Materials and methods

Plasmids

A retroviral vector containing c-Fos (pMVc-fos) has been described earlier [21]. To generate E-cadherin-expressing vectors, myc-tagged wild-type or mutant E-cadherin cDNA [46] was subcloned in pIRESpuro2 expression vector conferring puromycin resistance.

Retroviral infection

Infection of mouse epithelioid carcinoma cells with a pMVc-fos virus or with the empty vector, pMV-7, has been described previously [21]. Briefly, GP+E packaging cell line [47] was employed to produce replication-defective retroviruses. Virus-containing supernatant was used to infect MT1TC1, VMR-Liv or RAC10P cells using 4 μg/ml polybrene. Infected cell populations were selected for 10 days in the presence of 400 μg/ml G418.

Cell lines and transfections

Mouse mammary epithelioid adenocarcinoma cell lines MT1TC1 [48], VMR-Liv [49] and Rac10P [50], cells infected