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p53-Dependent repression of focal adhesion kinase in response to estradiol in breast cancer cell-lines

Suresh Anaganti [a], Lynnette Fernández-Cuesta [a], Anita Langerød [b], Pierre Hainaut [a], Magali Olivier [a,*]

[a] Molecular Carcinogenesis Group, International Agency for Research on Cancer, Lyon, France
[b] Department of Genetics, Institute for Cancer Research, Oslo University Hospital, Radiumhospitalet, Oslo, Norway

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ABSTRACT

Mutations in the *TP53* suppressor gene are frequent in breast cancers. These mutations are associated with poor prognosis, thought to be due to proliferative advantage and poor response to chemotherapy associated with loss of p53 function. The focal adhesion kinase (*FAK*/*PTK2*), a tyrosine kinase, is over-expressed in a variety of human tumors including breast cancers. *FAK* is a critical regulator of adhesion and motility and its over-expression is associated with increased metastatic potential. Recently, *FAK* promoter has been shown to contain p53 responsive elements and to be down-regulated by DNA-damage in a p53-dependent manner. Here, we have used five estrogen-dependent breast cancer cells lines with different p53 status, including an isogenic model, to show that *FAK* expression was regulated in a p53-dependent manner in response to estradiol. *FAK* protein and mRNA expression were down-regulated by estradiol in wild-type but not mutant p53 cells. Moreover, silencing wild-type p53 increased *FAK* expression, while over expressing p53 repressed *FAK* expression. ChIP experiment showed that p53 bound to *FAK* promoter in the presence of estradiol in p53 wild-type but not in mutant p53 cells, suggesting a direct role of p53 in down regulating *FAK* mRNA expression. FAK mRNA expression was also found to correlate with *TP53* mutation status in a series of breast tumors. Finally, loss of *FAK* down-regulation in p53 mutant cells was correlated with increased proliferation and invasion upon estradiol stimulation, while *FAK* silencing reduced invasion. These results suggest that p53 is an important down regulator of FAK and that loss of p53 function in breast cancer may contribute to the metastatic potential of estrogen-responsive tumors through uncontrolled *FAK* expression upon estrogens stimulation.

1. Introduction

The tumor suppressor gene *TP53* encodes the p53 protein, a stress-induced transcription factor that exerts anti-proliferative activities through the regulation of genes involved in the control of apoptosis, cell cycle checkpoints, senescence, DNA repair and metabolism [1]. In breast carcinomas, *TP53* somatic mutations are common, with 15–40% of tumors presenting an altered *TP53* gene, and are associated with aggressive disease and poor overall survival [2]. Although *TP53* mutations are less frequent in hormone receptors (HR) expressing tumors, we have previously observed that the prognostic value of *TP53* status is stronger in HR positive cases [3]. *TP53* germline mutations, which are causing the cancer susceptibility Li–Fraumeni syndrome, confer an increased risk of early breast cancer [4]. These observations imply an important role for p53 inactivation in mammary carcinogenesis.

Despite progresses in new therapeutic strategies, the prognosis of breast cancer patients with metastatic disease remains poor [5]. Invasion and metastasis requires that

* Corresponding author.
E-mail address: molivier@iarc.fr (M. Olivier).

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tumor cells alter their ability to interact with and adhere to both surrounding cells and the extracellular matrix (ECM). The integrin family of cell adhesion receptors play a critical role in these processes, consistent with their ECM binding properties [6,7]. Focal adhesion kinase (*FAK*/PTK2) is a non-receptor tyrosine kinase that is localized at the contact points between cells and their substratum [8], and associates with integrin receptors, recruiting other molecules to the site of this interaction. These interactions form a signaling complex that transmits signals from the extracellular matrix to the cytoskeleton, mediating growth-factor signaling that control cell proliferation, cell survival and cell migration [9,10]. Upon activation, *FAK* is auto-phosphorylated at Tyr397 creating a binding site for SH2 domain-containing proteins (such as c-Src kinase) leading to subsequent phosphorylation of other sites of *FAK* that maximizes *FAK* kinase activity [11]. In mice, deletion of *FAK* in the epidermis suppressed chemically induced skin tumor formation and blocked malignant progression [12]. In several types of human tumors, *FAK* expression is increased and has been proposed as potential therapeutic target in cancer [9,13]. In breast cancers, elevated *FAK* mRNA and protein levels were shown to be associated with invasive and metastatic disease [14,15], but the molecular mechanisms responsible for this increased expression remain largely unknown.

Two putative p53 binding sites have been identified in the human *FAK* promoter [16]. Recently, evidence for direct down-regulation of *FAK* by DNA-damage-activated p53 has been provided, and correlation between *FAK* protein expression and p53 mutations were significantly correlated in breast cancer samples [17,18]. These results suggest that inactivation of p53 could play a role in increased *FAK* expression in breast cancer.

In the present study, we investigate the possible regulation of *FAK* by p53 in conditions where p53 is not activated by DNA-damage but is stabilized by estrogens in hormone-dependent breast cancer cells. Our results provide evidence that in breast cancer cell-lines and breast tumors, loss of p53 function by mutation results in loss of *FAK* repression, which may contribute to increased proliferation and invasion in response to estradiol.

2. Materials and methods

2.1. Cell-lines and culture conditions

All cell-lines used in this study are listed in Supplementary Table S1. MN1 (p53 wild-type) and MDD2 (p53 inactivated) cells are isogenic cell-lines established in 1992 (kindly provided by M. Oren, Weizmann Institute), derived from MCF-7 cells. MDD2 cells are stably transfected with a pCMV-DD plasmid expressing a p53 mini protein that contains the first 14 and last 89 amino acids of the mouse p53 protein [19]. This mini p53 protein has strong dominant negative activity due to hetero-oligomerisation with endogenous p53 protein, resulting in the over-expression of an inactive p53 protein in MDD2 cells (p53 inactivated). MN1 cells were similarly generated by transfection of the control insert-free plasmid followed by G418 selection. MN1 and MCF-7 cells have a similar p53 response (not shown). MDA-MB-231 (p53 mutant, p.R280K) and MDA-MB-468 (p53 mutant, p.R273H) were acquired from Dr. Brautigan's lab (Center for cell signaling, University of Virginia, Charlottesville). These cell-lines were maintained in Dulbecco's Modified Eagle's Medium (DMEM) (Invitrogen) containing 10% fetal bovine serum (Perbio science, Belgium) 2% L-glutamine–penicillin–streptomycin solution stabilized (Sigma) and 0.4 mg/ml geneticin G418 (Invitrogen). ZR75-1 (p53 wild-type) and T47D (p53 mutant, p.L194F) cells were provided by Dr. Theillet and BT474 (p53 mutant, p.E285K) by Dr. Puisieux. These cells were maintained in RPMI medium 1640 (Invitrogen) containing 10% fetal bovine serum and 2% L-glutamine–penicillin–streptomycin solution stabilized (Sigma). MDA-MB-361 (p53 null, p.S166X) cells were acquired from cell culture core facility (University of Virginia, Charlottesville) and grown in Leibovitz's L-15 medium (GIBCO) containing 20% fetal bovine serum (GIBCO), 2% L-glutamine–penicillin–streptomycin solution stabilized (Sigma). All cells were cultured at 37 °C in a 5% CO_2 tissue culture incubator.

2.2. Breast tumor samples

Gene expression data obtained from a series of 80 breast tumor samples sequentially collected at the Ullevål University Hospital and previously reported in Langerod et al. [20] were analyzed. This dataset was already fully annotated for clinical variables, subtypes and *TP53* status. The relative expression level of one clone (IMAGE:855863, Stanford cDNA microarrays) corresponding to the PTK2 gene (FAK) was used for the current analysis.

2.3. 17β-Estradiol treatments

For all the experiments involving 17β-estradiol (E2) treatment cells were grown under estrogen-free (EF) condition for 48 h prior to treatment to remove endogenous steroid hormones that might interfere with the analysis. Cells were grown in phenol-red free DMEM/F12 for MN1 and MDD2, MDA-MB-231 and MDA-MB-468 cells, phenol-red free Leibovitz's L-15 medium for MDA-MB-361 cells or phenol-red free RPMI1640 for ZR75-1, BT474 and T47D, supplemented with 10% dextran charcoal-treated fetal bovine serum (Hyclone), 2% L-glutamine–penicillin–streptomycin solution stabilized (Sigma). 17β-estradiol (Sigma) dissolved in water was used at indicated concentrations in the appropriate EF medium.

2.4. Cell viability assays

Cell proliferation assays were performed in 96-wells plates using a MTS assay (CellTiter96R AQueousOneSolution, Promega) following the manufacturer's protocol. Absorbance at 490 nm was measured using a kinetic microplate reader (MRX Revelation, DYNEX Technologies). 1×10^4 cells were plated in each well at the start of the assay. For each treatment three independent experiments done in triplicates were performed.