Available online at www.sciencedirect.com

SCIENCE DIRECT

Matrix Biology 24 (2005) 469 – 477

MATRIX BIOLOGY

www.elsevier.com/locate/matbio

Type IV collagen induces STAT5 activation in MCF7 human breast cancer cells

Teresa Robledo [1], Lourdes Arriaga-Pizano [1], Mario Lopez-Pérez, Eduardo Pérez Salazar *

Departamento de Biología Celular, Cinvestav-IPN, México, DF. 07360 México

Received 23 March 2005; received in revised form 27 June 2005; accepted 25 July 2005

Abstract

A rapid increase in the tyrosine phosphorylation of signal transducer and activators of transcription (STAT) proteins has been extensively documented in cells stimulated with cytokines and growth factors, but virtually nothing is known about the regulation of STAT5 activation in breast cancer cells stimulated with basement membrane (BM) components. Stimulation of MCF7 cells with type IV collagen (Col-IV) promoted a striking increase in the phosphorylation of STAT5 at Tyr-694, as revealed by site-specific antibodies that recognized the phosphorylated state of this residue. In addition, Col-IV also stimulated STAT5 nuclear translocation and an increased in STAT5 DNA binding activity. Treatment with the selective Src family inhibitor pyrazolopyrimidine PP-2 prevented STAT5 phosphorylation at Tyr-694, nuclear translocation of STAT5 and the STAT5-DNA complex formation. Our results demonstrate, for the first time, that stimulation with Col-IV induces STAT5 phosphorylation of endogenous STAT5 at Tyr-694, nuclear translocation of STAT5 and increases in STAT5 DNA binding activity via a Src-dependent pathway in MCF7 cells.

Keywords: MCF7; Breast cancer; STAT5; Collagen; Extracellular matrix; Basement membrane

1. Introduction

Extracellular matrix (ECM), including basement membrane (BM), is a complex network of interacting molecules such as collagens, fibronectin, and laminin. This matrix, in addition to acting as a scaffold that stabilizes the physical structure of tissues, regulates vital cell processes (McClay and Ettensohn, 1987; McDonald, 1988). In normal mammary gland, BM is a continuous deposit that separates epithelial cells from the surrounding stroma. It is rich in laminin and type IV collagen (Col-IV), and also contains entactin, proteoglycans and other glycoproteins (Aumailley and Gayraud, 1998). Signals from BM regulate epithelial cell morphology, growth, differentiation and apoptosis in mammary cells (Boudreau et al., 1995; Muschler et al., 1999; Weaver et al., 2002).

Col-IV is a trimmer composed of three monomeric chains with the usual composition $(\alpha 1)_2(\alpha 2)$ and six isoforms have been described (Gunwar et al., 1998; Timpl et al., 1985). It is the major component of BM and provides the structural framework of all BM, interacting with cell surface biomolecules such as integrins and proteoglycans. BM promotes adhesion and motility of various normal or transformed cells and it is the first barrier that cells must traverse to produce metastasis (Miles et al., 1994; Wisdom et al., 1992; Yoshinaga et al., 1993).

The signal transducers and activators of transcription (STATs), originally identified in interferon signaling, can be

Abbreviations: Ab, antibody; BSA, bovine serum albumine; BM, basement membrane; Col-IV, type IV collagen; DMEM, Dulbecco's modified Eagle's medium; ECM, extracellular matrix; EGF, epidermal growth factor; EMSA, electrophoretic mobility shift assay; HEPES, 4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid; MMP, metalloproteinase; PAGE, polyacrylamide gel electrophoresis; PBS, phosphate-buffered saline; PP-2, pyrazolopyrimidine 2; PP-3, pyrazolopyrimidine 3; Rb, retinoblastoma; RIPA, radioimmune precipitation assay; STAT, signal transducers and activators of transcription; Tyr, tyrosine.

* Corresponding author. Tel.: +52 55 5061 3991; fax: +52 55 5747 7081.
E-mail address: jperez@cell.cinvestav.mx (E.P. Salazar).

[1] These authors contributed equally to this work.

0945-053X/$ - see front matter © 2005 Elsevier B.V./International Society of Matrix Biology. All rights reserved.
doi:10.1016/j.matbio.2005.07.004

activated by a number of cytokines, growth factors and ECM components (Brizzi et al., 1999; Cruz-Vera et al., 2003; Ihle et al., 1995; Schindler and Darnell, 1995). Activation of STATs involves phosphorylation of a single tyrosine at the C-terminus, hetero- or homo-dimerization, and translocation to the nucleus, where STATs bind to consensus elements in the promoter of genes that are regulated (Leaman et al., 1996; Liu et al., 1998). STAT proteins are normally involved in a variety of cellular processes including cell proliferation, differentiation, development, apoptosis and accumulating evidence supports a new role in oncogenesis (Bowman et al., 2000; Bromberg, 2001; Coffer et al., 2000).

In particular, STAT5 plays a key role in mammary epithelial cell growth and differentiation, as well as in prolactin-induced expression of milk protein genes, such as β-casein and β-lactoglobulin (Burdon et al., 1994; Gouilleux et al., 1994; Streuli et al., 1995; Wakao et al., 1994). Both isoforms of STAT5 (STAT5a and STAT5b, which share 93% identity at the amino acid level) can be activated by many cytokines, thrombopoietin, erythropoietin, epidermal growth factor (EGF) and platelet-derived growth factor in various cell lines and tissues, as well as by non-receptor tyrosine kinases such as Src and Bcr-Abl (Ilaria and Van Etten, 1996; Lange et al., 1998; Lin et al., 1995). Thus, STAT5 might play a variety of regulatory roles controlling different functions including cell growth, survival, and differentiation. Recent studies have demonstrated for example, that activated STAT5 may regulate cyclin D1 promoter activity resulting in cell cycle progression (Matsumura et al., 1999; Schroeder et al., 2002). Nevertheless, the role of STAT5 in breast cancer has not been established, although significant elevations in the DNA binding activity of both STAT3 and STAT5 are found in malignantly transformed breast tissues when compared to normal tissues and a dominant-negative STAT5 suppresses transcriptional activity of estrogen receptors and induces apoptosis in T47 breast cancer cells (Watson, 2001; Watson and Miller, 1995; Yamashita et al., 2003, 2004).

In the present study, we report that stimulation with Col-IV induces phosphorylation of endogenous STAT5 at Tyr-694, nuclear translocation of STAT5 and an increase in STAT5-DNA complex formation in MCF7 cells. Treatment with the selective Src kinase inhibitor PP-2 inhibited all these effects induced by Col-IV. Our results demonstrate, for the first time, that the signaling events leading to STAT5 activation induced by Col-IV are Src-dependent in MCF7 cells.

2. Results

2.1. Col-IV stimulation induces STAT5 phosphorylation at Tyr-694 and nuclear translocation of STAT5 in MCF7 cells

In cancer, activation of STATs is frequently observed, and it is postulated that dysregulation of these factors may be involved in its pathogenesis (Bowman et al., 2000; Bromberg, 2001). In order to determine whether Col-IV induces STAT5 phosphorylation at Tyr-694 in MCF7 cells, cells placed in suspension were plated onto dishes coated with either Col-IV or poly-L-lysine for various times and lysed. Cell lysates were analyzed by SDS–PAGE followed by Western blotting with a phosphospecific Ab against the Tyr-694 of STAT5 (anti-STAT5-Tyr(P)^{694} Ab). As shown in Fig. 1A (upper panel), treatment of cells with Col-IV induced a marked increase in STAT5 phosphorylation at Tyr-694 that could be detected within 15 min, reached a maximum between 15 and 20 min, and declined toward base-line levels after 60 min of treatment. Western blotting with an Ab anti-STAT5 C-17, which recognizes the C-terminal sequence of STAT5a and STAT5b, of the same membranes confirmed that similar amounts of STAT5 protein were recovered after plating onto ligand-coated dishes (Fig. 1A, lower panel).

Upon receptor activation, STAT5a and/or STAT5b are tyrosine phosphorylated and dimers of STAT5 are formed with subsequent translocation to the nucleus (Buitenhuis et al., 2004). To examine whether Col-IV induces nuclear translocation of STAT5, MCF7 cells placed in suspension were plated onto either Col-IV or poly-L-lysine for various times and nuclear extracts were obtained. Nuclear extracts were analyzed by Western blotting with anti-STAT5 Ab C-17 or with anti-retinoblastoma (Rb) Ab C-15 as the loading control. Our results show that in untreated cells STAT5 was not found in the nucleus. As expected, Col-IV promoted nuclear translocation of STAT5 in a time-dependent manner, which could be detected within 15 min and reached a maximum between 15 and 20 min (Fig. 1B).

These results indicate that Col-IV induces a selective increase in the phosphorylation of endogenous STAT5 at Tyr-694 and STAT5 nuclear translocation in MCF7 cells. Taken together, these results strongly suggest that Col-IV promotes STAT5 activation, formation of dimers and an increase in DNA binding activity.

2.2. Induction of STAT5 DNA binding activity by Col-IV

As already stated, STAT dimers once in the nucleus bind to specific DNA sequences present in the promoter-enhancer regions of target genes. To substantiate further that Col-IV promotes STAT5 activation with subsequent binding to DNA, we determine whether Col-IV treatment induces DNA binding activity of STAT5. MCF7 cells were treated with either Col-IV or poly-L-lysine for various times and nuclear extracts were obtained. EMSAs were then performed using nuclear extracts and a radiolabeled oligonucleotide probe representing a canonical STAT5 binding site. As illustrated in Fig. 2, a very weak specific STAT5-DNA complex was observed in unstimulated cells, however the complex formation significantly increased in a time-dependent manner in cells stimulated with Col-IV and persisted longer than 60 min. The specificity of these