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Negative regulation of interferon-γ/STAT1 signaling through cell adhesion and cell density-dependent STAT1 dephosphorylation

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ABSTRACT

Signal transducer and activator of transcription 1 (STAT1) is an important mediator for cytokine signal transduction, particularly IFN-γ. Following IFN-γ stimulation, STAT1 is activated through tyrosine phosphorylation. Little is known about the function and regulation of STAT1 dephosphorylation after activation. We studied the regulation and function of STAT1 dephosphorylation in different types of cells and found that the phosphorylated STAT1 was quickly dephosphorylated in most of epithelial cells. Further studies revealed that the dephosphorylation of STAT1 was regulated by cell shape/adhesion. Actin cytoskeleton and extracellular matrix (ECM) proteins mediated the STAT1 dephosphorylation through the T-cell protein tyrosine phosphatase TCPTP. Inactivation of the dephosphorylation system by cell detachment rendered the cells more sensitive to IFN-γ-induced cell death. Our results revealed a novel mechanism in regulating IFN-γ/STAT1 signaling. This cell adhesion and cell cytoskeleton-dependent STAT1 dephosphorylation system may have a role in IFN-γ-mediated immunosurveillance for cancer cells by inducing anoikis of detached metastatic cancer cells.

1. Introduction

IFN-γ was discovered more than 35 years ago as an anti-viral agent [1]. It has subsequently been found to have many important functions including inhibiting tumor cell growth [2–4], regulating immune responses [5–7], and inducing cell differentiation [8,9]. Signal transducer and activator of transcription 1 (STAT1), which is the major mediator of IFN-γ signaling, is a member of a family of seven transcription factors that are activated by different types of cytokines [10,11]. Upon IFN-γ, or other cytokines or growth factors, stimulation, STAT1 is activated through tyrosine phosphorylation by cytokine-receptor associated JAK kinases or the respective growth factor receptor tyrosine kinases at the plasma membrane raft [10–13]. It then departures from the membrane receptor and moves into cytoplasmic soluble multiprotein chaperone complexes and dimerizes in association with these complexes, followed by nuclear importing and binding to its target genes [14,15]. Serine phosphorylation, by itself or in addition to tyrosine phosphorylation [16], and arginine methylation further modulate the cellular localization and function of the STAT proteins [17]. Numerous studies have been done to delineate these signaling events leading to the expression of the STAT1-targeted genes. However, the fate and regulation of STAT1 protein after gene activation are much less studied.

It usually takes 5–60 min for STAT1 activation and nuclear import after IFN-γ stimulation in cultured cells [18]. Inactivation of the tyrosine-phosphorylated STAT1 takes place 2–4 h after cytokine addition in most cells and involves dephosphorylation in both the nuclear and the cytoplasmic compartments [19]. Dephosphorylated STAT1 proteins in the nucleus are cycled back to the cytoplasm [20]. Protein tyrosine phosphatases, particularly the T-cell protein tyrosine phosphatase (TCPTP), have been reported to be involved in the inactivation cycle of STAT1 in IFN-γ treated cells [19]. It was speculated that the TCPTP was involved in the dephosphorylation of STAT1 in both nucleus and cytoplasm because it exists in both places [19]. Instead of a simple enzyme–substrate reaction, STAT1 dephosphorylation seemed to be an exquisite process strictly regulated at different levels. It was reported that STAT1 required a N-terminal domain-mediated conformational change to undergo dephosphorylation by TCPTP [21]. Recently, the nuclear beta-arrestin1 was shown to function as a scaffold of TCPTP-STAT1 dephosphorylation complex [22].

TCPTP itself was reported to be regulated by an auto-inhibition mechanism. In vitro studies, using proteolytically cleaved fragments of TCPTP, have demonstrated that the catalytic activity of TCPTP is regulated by an intramolecular inhibition involving a carboxy-terminal segment of the 45 kD form of TCPTP [23]. It was found that association of TCPTP with integrin-alpha1 following cell adhesion to

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extracellular matrix could alleviate this auto-inhibition and lead to activation of the phosphatase activity [24]. Therefore, it appears that both STAT1 and TCPTP are regulated by conformational changes. The molecules and mechanisms that mediate the conformational changes however have not been revealed.

STAT1 is expressed in immune as well as in non-immune cells. The functions and regulations of STAT1 in immune cells are well-studied. STAT1 mainly mediates IFN-γ signal to regulate immune cell activation [5,7,25–28]. The functions and regulations of STAT1 in non-immune cells are less studied, although it has been reported that STAT1 acts as a tumor suppressor in many cancer cells [29–31].

During our study on the regulation and function of STAT1 in different types of cells, we found that the tyrosine dephosphorylation of STAT1 after stimulation and activation was more significant in epithelial cells. The dephosphorylation of STAT1 was mediated by an ECM/actin-cytoskeleton-dependent mechanism, which rendered the STAT1 activation as a transient event in the epithelial cells. Inactivation of this dephosphorylation system upon cell detachment from their surrounding matrix environment may render the cells more sensitive to IFN-γ-induced anoikis. Defects in this regulatory system may results in cell death or cancer.

2. Materials and methods

2.1. Cells and reagents

A HepG2 cell line stably transfected with a GAS-luciferase reporter gene was obtained from Dr. X. Fu (Indiana University, Bloomington, IN). HGC (human gastric carcinoma cell), HeLa and H4 (human neuroglioma cell) cells were maintained in DMEM medium (GIBCO) supplemented with 10% FCS (Hyclone). HepG2 cells were cultured in α-MEM (GIBCO) with 10% FCS. U937, Jurkat and HL-60 cells were maintained in RPMI 1640 (GIBCO) with 10% FCS.

Collagen was purchased from Shengyou Bioscience; fibronectin was from Bosheng Bioscience; cytochalasin D was from Tocris Bioscience; nocodazole was from Sigma-Aldrich; human IFN-γ was

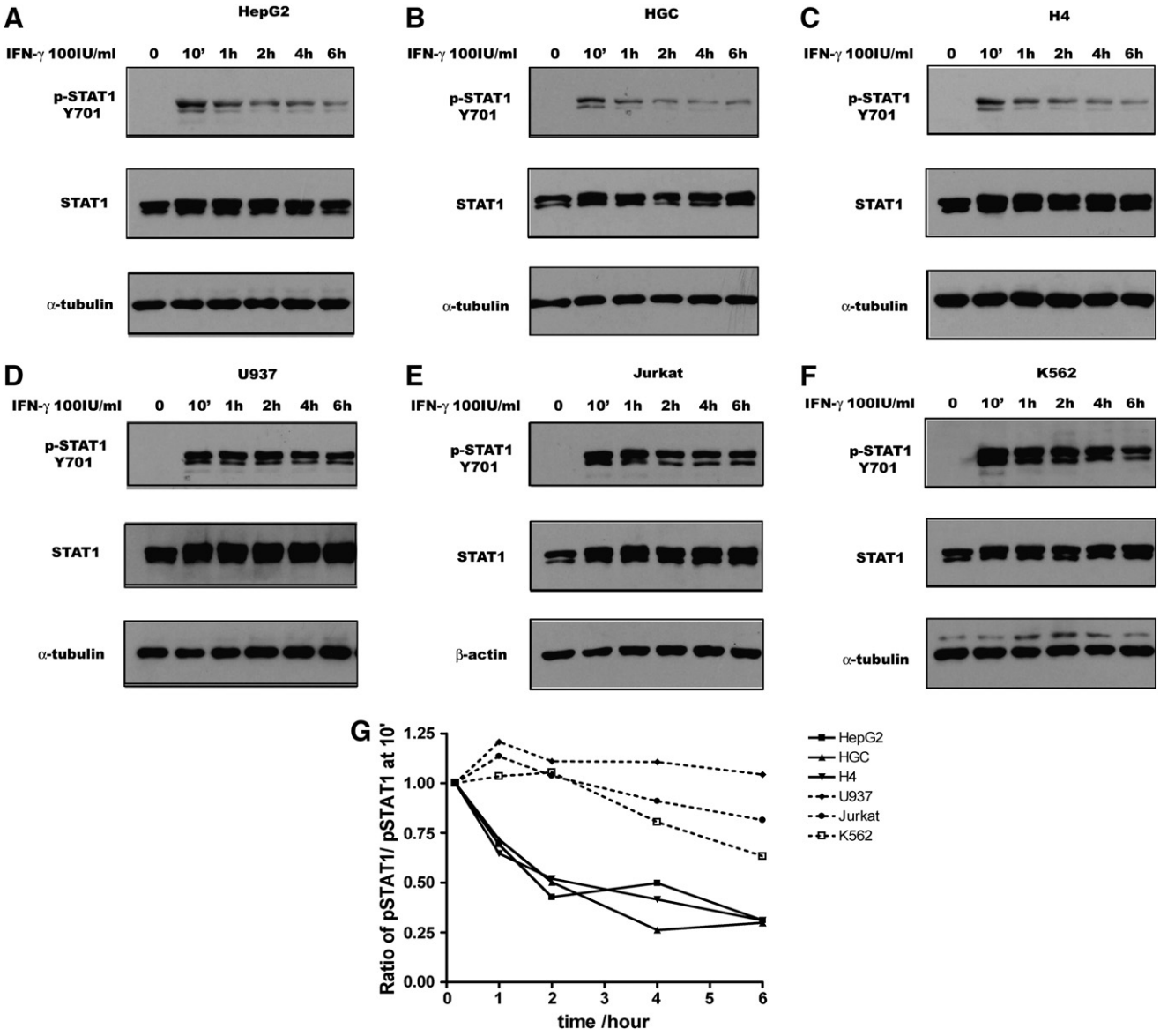

Fig. 1. STAT1 dephosphorylation in non-immune cells and immune cells. Non-immune cells HepG2, HGC, and H4 (A–C) and immune cells U937, Jurkat, and K562 (D–F) were stimulated with INF-γ for different time periods as indicated. The cell lysates were analyzed by immunoblotting using anti-pY701-STAT1 or anti-STAT1 antibody. Anti-α-tubulin or β-actin antibody was used as a loading control. (G) Graphs of densitometric quantifications of the ratios of pY-STAT1 at different time periods to pY-STAT1 at 10 min in A–F were normalized to total STAT1.