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Cleavage of focal adhesion kinase is an early marker and modulator of oxidative stress-induced apoptosis

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Abstract

Focal adhesion kinase (FAK) is a signaling molecule associated with cell survival. Previously, we showed that thimerosal, a reactive oxygen species (ROS) generator, can acutely induce FAK tyrosine phosphorylation (within minutes) and chronically induce apoptosis (within days) by redox modulation in HeLa S cells. In the present study, we report that a prolonged oxidative stress by thimerosal induces a remarkable cleavage of FAK, which is accompanied with apoptosis. In fact, the kinetics of FAK cleavage has a good correlation with and actually preceding the apoptosis that was independent of anoikis. The effects were almost completely blocked by the pretreatment with either *N*-acetyl-L-cysteine (ROS scavenger) or Z-VAD-FMK (pan-caspase inhibitor), suggesting ROS-induced caspase activation as a key mechanism. They could be also reproduced by hydrogen peroxide alone, which appeared to be responsible for thimerosal-mediated oxidative stress-induced apoptosis. Additionally, the down regulation of FAK with antisense oligonucleotide dramatically augmented thimerosal-induced apoptosis. We could observe similar results using human corneal epithelial cells. Taken together, our results show that FAK is a critical cellular target of caspases during oxidative stress (particularly by hydrogen peroxide), resulting in the acceleration of subsequent apoptosis regardless of the anchorage status of cells. From the present results, it is more likely that not cell detachment but the proteolytic cleavage (or inhibition) of FAK is a key modulator as well as a promising indicator of apoptosis in epithelial cells under oxidative stress.

Keywords: Oxidative stress; Thimerosal; Caspase; Focal adhesion kinase; Proteolysis; Apoptosis

1. Introduction

Cellular reduction–oxidation (redox) state is primarily a consequence of the precise balance between the levels of reactive oxygen species (ROS) and endogenous thiol buffers present in the cell, including glutathione and thioredoxin, which protect cells from oxidative damage. Disturbance of cellular redox status, such as a ROS generation that exceeds compensatory changes in the

Abbreviations: FAK, focal adhesion kinase; ROS, reactive oxygen species; NAC, *N*-acetyl-l-cysteine; TM, thimerosal; TSA, thiosalicylic acid; Genis, genistein; Herbim, herbimycin A; HCE, human corneal epithelial; PTK, protein tyrosine kinase

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level of the endogenous thiol buffers, may result in the sustained activation of signaling pathways and expression of genes that induce apoptosis [1]. ROS-induced apoptosis has been previously demonstrated in various cell types. Transforming growth factor beta (TGF-β)-induced apoptosis in fetal hepatocytes is mediated by mitochondrial-dependent ROS generation [2]. However, redox-dependent apoptosis appeared to be a highly complex process. Tumor necrosis factor alpha (TNF-α)-induced endothelial cell apoptosis is inhibited by Rac-1 dependent ROS but promoted by mitochondrial-derived ROS, suggesting the dual roles of ROS in the apoptosis [3]. Diamide, a thiol oxidant, induces apoptosis in mitotic competent undifferentiated cells by cellular redox imbalance, which is independent of ROS production [4]. It is even paradoxical that antioxidant resveratrol, a tumor-suppressive compound, can generate ROS and induce apoptosis, which is independent of cytochrome *c* release [5]. Thimerosal is a preservative widely used in biological products, including vaccines, cleaning solutions for contact lenses, and cosmetics since the 1930s. However, there have been several reports that thimerosal has potential side effects, including causing inflammatory diseases [6,7]. Previously, we showed that thimerosal can generate ROS, including hydrogen peroxide, thereby acutely (within minutes) stimulates FAK tyrosine phosphorylation and cytoskeletal rearrangement [8]. However, prolonged (24 h) exposure to thimerosal resulted in apoptosis of HeLa S cell by redox modulation [9]. Empirically, the ROS generation appears to have a central role and cause many (if not most) of the cellular changes in thimerosal treated cells.

Focal adhesion kinase (FAK) is a nonreceptor protein tyrosine kinase (PTK) and a key mediator of integrin signaling, which implicates its regulatory roles in cell adhesion, spreading, migration as well as cell survival and proliferation [10–12]. Proteolytic cleavage of FAK has been reported in various cell types, including c-Myc-induced apoptosis of chicken embryo fibroblasts (CEF) [13], growth factor deprivation-induced apoptosis of human umbilical vein endothelial cells [14]. Especially, the ordered proteolytic cleavage of FAK has been well described in detachment-induced cell death (anoikis) of intestinal epithelial cells [15]. They showed that the first cleavage by caspase-3 generated a 94/92-kDa terminal fragment, which was subject to the second cleavage by caspase-6 to an 84-kDa fragment. However, the significance of FAK cleavage in apoptotic process has not been properly evaluated and its role is still ambiguously understood. In the present study, we propose that FAK is a critical target of oxidative stress-activated caspases, which in turn accelerates subsequent apoptotic cell death regardless of the anchorage status of cells. For this, we used HeLa S cell, since it can normally grow and proliferate either in anchorage culture (with triangular type morphology) or in suspension culture (with spherical type morphology) depending on the kinds of culture dishware used, either a regular cell culture dish or a Petri dish, respectively. The use of HeLa S cells allowed us to exclude a possibility of anoikis-type apoptosis and judge the genuine impact of FAK cleavage in apoptotic process. When the cells were treated with a ROS generator, thimerosal, they showed membrane protrusions, rounding up, and FAK cleavage, thereby resulting in anoikis-like apoptosis. However, the cell death was not an anoikis since HeLa S cells do not go anoikis, but it was rather related to FAK cleavage. From this study, we propose that not cell detachment but the proteolytic cleavage (or inhibition) of FAK is a key modulator and a promising indicator of apoptosis in epithelial cells under oxidative stress.

2. Materials and methods

2.1. Chemicals and reagents

Thimerosal (Mercury-[(*o*-carboxyphenyl) thio]-ethyl sodium salt), *N*-acetyl-L-cysteine, Hoechst 33342, and (3-[4,5-dimethylthiazol-2-yl]-2,5-di-phenyl) tetrazolium bromide (MTT) were purchased from Sigma (St. Louis, MO, USA). Thiosalicylic acid was purchased from Aldrich Chem. Co. (Milwaukee, WI, USA). BAPTA-AM was obtained from Molecular Probes Inc. (Eugene, OR, USA). Protein tyrosine kinase inhibitors, herbimycin A and genistein were purchased from Calbiochem (La Jolla, CA, USA). Trypan blue was obtained from Gibco BRL (Grand Island, NY). Z-VAD-FMK (*n*-benzyloxycarbonyl-Val-Ala-Asp(*O*-Me) fluoromethyl ketone) was obtained from Enzyme Systems Products (Livermore, CA, USA). Mouse monoclonal anti-FAK antibody was purchased from Transduction Laboratories (Lexington, KY, USA). Rabbit polyclonal anti-Pyk2 antibody was obtained from Upstate cell signaling solutions (Charlottesville, VA, USA). Rabbit monoclonal anti-caspase-3 antibody was purchased from Cell Signaling Technology (Danvers, MA, USA). Monoclonal anti-GAPDH antibody (clone 6G5) was purchased from Biogenesis Ltd. (England, UK).

2.2. Cell culture

HeLa S cells were maintained in DMEM (Biowhittaker, Walkersville, MD, USA) supplemented with 10%