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Ionizing radiation can induce GSK-3β phosphorylation and NF-κB transcriptional transactivation in ATM-deficient fibroblasts

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Abstract

DNA damage by ionizing radiation (IR) can induce activations of both NF-κB and p53 through the upstream kinase ataxia telangiectasia mutated (ATM). NF-κB activation could also be signaled through two distinct or overlapped pathways; IκB kinases (IKKs)-IκBα and Akt–glycogen synthase kinase-3 (GSK-3). In the present study, however, we show that activation of Akt1 and the subsequent phosphorylation and inactivation of GSK-3β by IR could also occur in ATM-deficient AT5BIVA cells as well as in normal MRC5CV1 fibroblasts. Similarly, lithium chloride (LiCl) was found to increase the phosphorylation of GSK-3β independently of ATM. Transfection with either wild-type or kinase dead mutant GSK-3β to the cells further indicated that phosphorylations of Akt1 and GSK-3β were closely associated with the transcriptional transactivation of NF-κB in response to ionizing radiation. On the other hand, LiCl, having no effect on caspase-3 activation, significantly increased p53 phosphorylation and apoptotic death of the normal MRC5CV1 cells while IR, activating both caspase-3 and p53, profoundly affected AT5BIVA cell death. Hence, our data suggest that although ATM-mediated IKK–IκBα pathway might be a typical pathway for IR-induced NF-κB activation, GSK-3β phosphorylation could also partially contribute to the transcriptional transactivation of NF-κB in an ATM-independent manner and that GSK-3β phosphorylation could induce ATM-mediated cell apoptosis through the activation of p53.

Keywords: Akt; GSK-3; Ataxia telangiectasia; Ionizing radiation

1. Introduction

Exposure of eukaryotic cells to multiple forms of environmental stresses, including ionizing radiation (IR), gives rise to a variety of cellular responses such as DNA repair, gene induction, cell cycle arrest, apoptosis, and lethality [1,2]. IR-induced signaling is well known to proceed through the radiation sensor protein, ataxia telangiectasia mutated (ATM). ATM protein kinase is a master regulator of the cellular response to DNA lesion [3,4]. ATM is missing or inactivated in patients with the autosomal recessive disease ataxia telangiectasia (A-T). Cells from patients suffering from A-T are hypersensitive to ionizing radiation and show multiple defects in activating the cell cycle checkpoints in response to IR-induced DNA damage, characterized by cerebellar ataxia, oculocutaneous telangiectasia, immunodeficiency, genomic instability, cancer predisposition, and extreme sensitivity to IR and radiomimetic chemicals [5–7].

Ionizing radiation has been reported to induce NF-κB activation following small and large doses [8,9], and it is well known that double strand breaks (DSBs) by DNA damaging agent, camptothecin (CPT), can induce ATM-dependent NF-κB activation [10]. NF-κB family includes p65/RelA, p50/NF-κB1,

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p52/NF-κB2, c-Rel and RelB [10,11]. NF-κB is involved not only in inflammatory diseases and oncogenesis but also in apoptotic processes induced by cytokines and anti-tumor agents [12,13]. Among the factors associated with NF-κB activation, Akt, a family of serine/threonine kinase, was reported to stimulate IKK to phosphorylate and activate the p65 subunit of NF-κB [14], controlling the expression of several anti-apoptotic proteins such as FLIP, XIAP, surviving, and IAP-2 [15]. Moreover, involvement of Akt1 in regulation of transcriptional transactivation of NF-κB has also been reported [16], implicating the existence of different mechanisms of Akt regulation of NF-κB.

Glycogen synthase kinase-3β (GSK-3β) is a ubiquitously expressed protein serine/threonine kinase, emerging as a regulator of neuronal, endothelial, hepatocyte, fibroblast, and astrocyte death [17,18]. GSK-3β is also involved in diverse cellular processes, including glycogen synthesis, proliferation, apoptosis, and development [19]. Stimulation of cells with insulin causes inactivation of GSK-3β through a PI3K/Akt-dependent mechanism [20]. Phosphorylation of GSK-3β by Akt has also been reported in irradiated vascular endothelium [21]. On the other hand, mice with inactivated GSK-3β died from hepatocyte apoptosis during development due to a defect in NF-κB activation [22]. Likewise, lithium chloride (LiCl), an established inhibitor of GSK-3, sensitized primary rat hepatocytes toward TNFα-mediated apoptosis resulting in 90% cell death after 24 h, accompanied by increased caspase-8 and caspase-3 activities and typical DNA laddering [23]. In respect of transcriptional control, regulation of phosphorylation and function of CCAAT/Enhancer-binding protein β (CEBPβ) by Akt and GSK-3 was described in growth hormone-treated 3T3-F442A fibroblasts [24]. Transcriptional activation by heat shock factor-1 was shown to be repressed by GSK-3 phosphorylation [25]. Moreover, phosphorylation and inhibition of rat glucocorticoid receptor transcriptional activation by GSK-3 was also reported [26]. Despite the accumulating data on the regulatory roles of GSK-3 in diverse cellular signalings, however, the exact function of GSK-3 phosphorylation in relation with ATM, NF-κB activation and cell growth has not yet been clearly defined.

Previously, it was reported that activation of Akt in response to insulin or IR was mediated through ATM [27]. Recently, however, we found an increased degradation of Akt1 in A-T fibroblasts exposed to a DNA damaging agent, camptothecin [28]. In our present investigation, we found an unexpected result showing that phosphorylation of Akt and GSK-3 could also occur in A-T as well as in normal cells, mediating the transcriptional transactivation of NF-κB in response to IR. Although not clearly identified yet, the crucial role of GSK-3 phosphorylation seems to be closely associated with the phosphorylation of the tumor suppressor p53 in an ATM-dependent manner. Thus, we present new data demonstrating

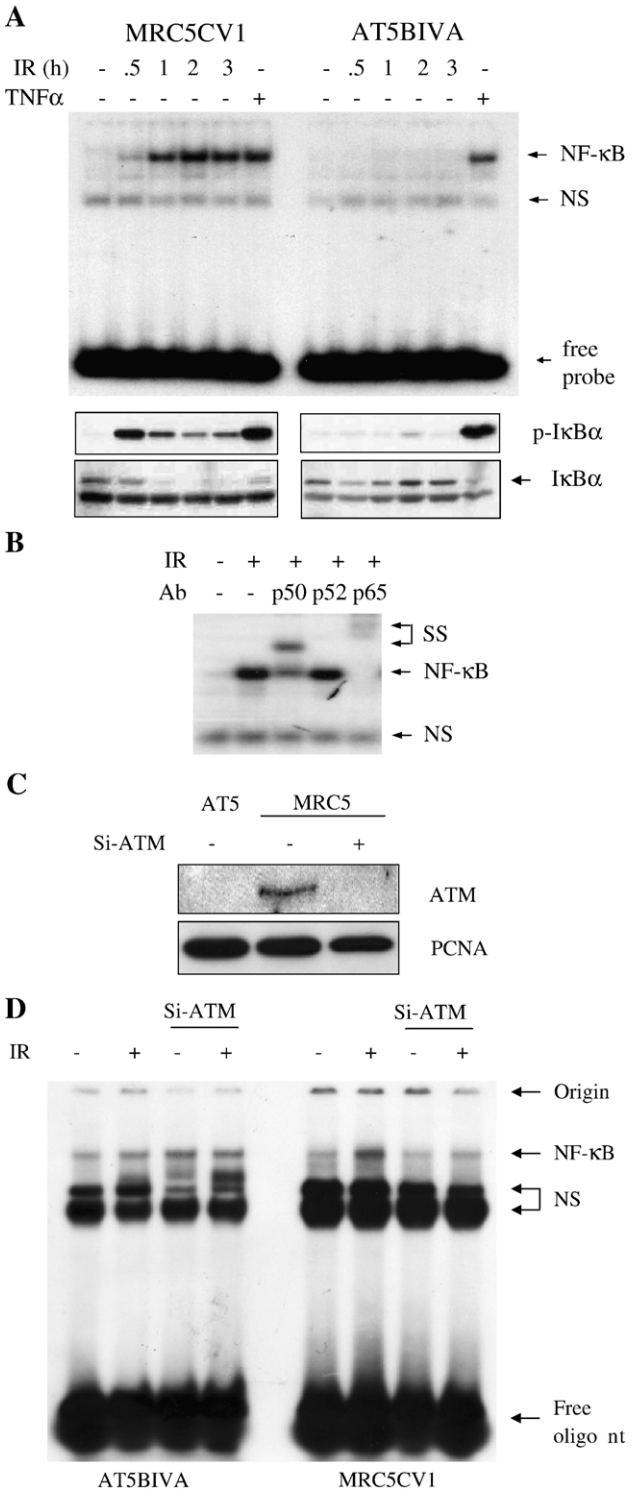

Fig. 1. Differential activation of NF-κB in AT5BIVA and MRC5CV1 cells in response to ionizing radiation. (A) EMSA analysis for NF-κB activation. Cells serum starved overnight were exposed to ionizing radiation (20 Gy) or challenged to TNFα (10 ng/ml, 15 min). At appropriate time points, cells were placed on ice and treated with ice-cold lysis buffer for cytosolic or nuclear fractionation as described in Materials and methods. Equal amount (10 μg) of the nuclear fractions was subjected to EMSA analysis. NS; non-specific binding. (B) Supershift analysis for the determination of the NF-κB complex. Each of the specific antibodies to p50, p52 and p65 was premixed with nuclear lysate on ice for 30 min followed by the addition of radioisotope-labeled NF-κB oligonucleotides. All the remaining procedures were done according to the methods as described in Materials and methods. NS; non-specific binding. (C) Repression of ATM expression by SiRNA. Cells were transfected with SiATM for 48 h and the nuclear fraction obtained for immunoblot analysis with an antibody to ATM. (D) EMSA analysis for IR-induced NF-κB activation after transfection of SiATM. Cells transfected with SiATM as in (C) were lysed and the nuclear fractions subjected to EMSA analysis.