Cancer Cell
Article

Regulation of XIAP Translation and Induction by MDM2 following Irradiation

Lubing Gu,[1] Ningxi Zhu,[1] Hongying Zhang,[1] Donald L. Durden,[1] Yue Feng,[2] and Muxiang Zhou[1,*]
[1]Department of Pediatrics
[2]Department of Pharmacology
Aflac Cancer Center and Blood Disorders Service, Emory University School of Medicine, Atlanta, GA 30322, USA
*Correspondence: mzhou@emory.edu
DOI 10.1016/j.ccr.2009.03.002

SUMMARY

Increases in protein levels of XIAP in cancer cells have been associated with resistance to apoptosis induced by cellular stress. Herein we demonstrate that the upregulation of XIAP protein levels is regulated by MDM2 at the translational level. MDM2 was found to physically interact with the IRES of the XIAP 5′-UTR, and to positively regulate XIAP IRES activity. This XIAP IRES-dependent translation was significantly increased in MDM2-transfected cells where MDM2 accumulated in the cytoplasm. Cellular stress and DNA damage triggered by irradiation induced the dephosphorylation and cytoplasmic localization of MDM2, which also led to an increase in IRES-dependent XIAP translation. Upregulation of XIAP in MDM2-overexpressing cancer cells in response to irradiation resulted in resistance of these cells to radiation-induced apoptosis.

INTRODUCTION

Inhibitor-of-apoptosis proteins (IAP) form a family of caspase inhibitors that block the downstream portion of the apoptosis pathway and inhibit cell death in response to multiple stimuli. There are currently eight known human IAP family members, and XIAP is a very important inhibitor of apoptosis among these members (Schimmer, 2004; Eckelman et al., 2006). XIAP has been shown to bind specifically to and inhibit caspases 3, 7, and 9, but not the remaining caspases (Riedl et al., 2001; Shiozaki et al., 2003). The distinct cascades of caspase activation regulate different apoptosis pathways that can be triggered via intrinsic and extrinsic signals (Green, 2005). For example, activation of caspase-9 initiates the intrinsic (mitochondrial) pathway of apoptosis, which is the major mechanism involved in cell death induced by cellular stress and DNA damage, such as from exposure to ultraviolet irradiation (UV) and ionizing radiation (IR) (Kuida et al., 1998). Activation of caspases 3 and 7 are distal steps in this intrinsic apoptosis pathway (Lakhani et al., 2006). The specific inhibition of these caspases by XIAP suggests that XIAP is a molecule that is critical for regulating sensitivity to apoptosis induced by cellular stress and DNA damage.

Previous studies have demonstrated that the expression of XIAP mRNA in most tissues and cells is fairly consistent (Liston et al., 1996). Cellular stresses such as exposure to IR will induce alteration of XIAP protein expression, without concomitant changes in XIAP mRNA levels, in a variety of cancer cells (Holcik et al., 1999). These observations suggest that the expression of XIAP is mainly regulated at the translational level. Initiation of translation can occur by two distinct mechanisms, cap-dependent scanning and internal ribosome entry. The latter mechanism requires an internal ribosome entry segment (IRES) located in the 5′ untranslated region (UTR) of the mRNA and the interaction of IRES *trans*-acting factor (ITAF)/ribonucleoprotein (RNP) (Stoneley and Willis, 2004). So far, IRES elements have been found mainly in mRNAs involved in regulating gene expression during development, differentiation, cell growth, and survival (Bonnal et al., 2003). In particular, IRES of certain antiapoptotic genes become activated under conditions where cap-dependent protein synthesis is greatly reduced, such as upon cellular stress and DNA damage; thus, activated IRES initiates translation of proteins that can protect cells from stress (Komar and Hatzoglou, 2005). It is known that XIAP translation is uniquely regulated by the IRES mechanism. There is a 162-nucleotide (nt)

SIGNIFICANCE

Overexpression of the MDM2 oncoprotein, which is often found in cancer cells, is associated with resistance to chemoradiation therapy and poor prognosis of cancer patients. MDM2 is well characterized as an inhibitor of the tumor suppressor p53, and overexpression of MDM2 contributes to a growth advantage for cancer cells. However, the mechanism by which overexpression of MDM2 confers resistance to DNA damage induced by irradiation and chemotherapy is not fully elucidated. We report here that MDM2 was able to bind to XIAP mRNA and positively regulate the IRES-dependent XIAP translation during cellular stress triggered by irradiation. These results identify a p53-independent function for MDM2 in mediating XIAP translation, which is critical in effecting cancer cell response to chemoradiation therapy.

IRES sequence in the 5′-UTR of XIAP mRNA (Holcik et al., 1999). Three ITAFs/RNPs, namely La, hnRNP C1/C2, and hnRNP A1, have been identified as able to regulate XIAP IRES activity (Holcik and Korneluk, 2000; Holcik et al., 2003; Lewis et al., 2007). Reports show that IRES-regulated XIAP translation is activated in cancer cells in response to IR, and that upregulation of XIAP results in increased resistance to apoptosis induced by this stress stimulation (Holcik et al., 2000; Lewis and Holcik, 2005).

The MDM2 protein is a multifunctional oncoprotein, and its ability to inactivate the p53 function through interaction with this tumor suppressor has been well characterized. In addition to interacting with and regulating p53, it has been demonstrated that MDM2 interacts with other molecules including specific protein and RNA, which might play a p53-independent role in oncogenesis. For example, MDM2 was shown to bind to and ubiquitinate Rb, resulting in Rb degradation and release of the E2F1 that promotes cell cycle progression (Xiao et al., 1995). MDM2 was also found to bind E2F1 directly, and to enhance E2F1 stability (Zhang et al., 2005). The C-terminal RING finger domain of MDM2 was found to exhibit specific RNA binding ability. A SELEX (systematic evolution of ligands by exponential enrichment) procedure yielded a subset of RNA molecules that bind efficiently to MDM2 in vitro (Elenbaas et al., 1996).

Aside from regulating other cellular molecules, MDM2 itself is modulated by various cellular signals. Phosphorylation and subcellular distribution of MDM2 is regulated by the PI3K/Akt pathway (Mayo and Donner, 2001). Cell growth/survival factor-induced activation of PI3K and its downstream target Akt will phosphorylate cytoplasmic MDM2 on serines 166 and 186. Phosphorylation of these sites is required for translocation of MDM2 from the cytoplasm into the nucleus. In contrast to survival signals that induce MDM2 phosphorylation, cellular stress and DNA damage invoke dephosphorylation of MDM2 (Meek and Knippschild, 2003; Blattner et al., 2002). It has been shown that dephosphorylation of the central acidic domain of MDM2 is essential for accumulation and stabilization of p53 in stressed wild-type (wt) p53 cells. Stress also induces signaling for dephosphorylation of MDM2 at serine 166 (Okamoto et al., 2002), which might lead to inhibition of nuclear entry of MDM2 or an increase in its translocation from the nucleus to the cytoplasm. Once MDM2 is released from p53 and is localized in the cytoplasm, it could play a p53-independent role. Because MDM2 is able to bind RNA and shuttles between the nucleus and the cytoplasm, which are the properties of most ITAFs/RNPs, we hypothesize that the dephosphorylated cytoplasmic MDM2 might act as an ITAF/RNP to exhibit a p53-independent role in regulating translation through binding of its C terminus to specific RNA.

We have previously demonstrated a link between MDM2 and XIAP. In a study utilizing transfection of MDM2 into a p53-null leukemia cell line, we found that the expression of XIAP protein is upregulated in the MDM2-transfected cells (Gu et al., 2002). Because the expression of XIAP is primarily regulated at the translational level though the IRES-dependent mechanism, we have now investigated the possible regulation of XIAP translation by MDM2 and the mechanism by which MDM2 acts as an ITAF/RNP to regulate XIAP IRES activity. Furthermore, we investigated the impact of MDM2-mediated XIAP expression on irradiation-induced apoptosis in human cancer cells.

RESULTS

IR Induces MDM2 Modulation and XIAP Translation

It is known that IR treatment dephosphorylates MDM2. To test whether IR-induced dephosphorylated MDM2 becomes dissociated from the MDM2-p53 complex and translocated from the nucleus to the cytoplasm, we examined the cellular redistribution of MDM2 and its association with p53 following IR treatment in an acute lymphoblastic leukemia (ALL) cell line EU-1. This cell line was chosen for the test because EU-1 cells have a wt p53 and overexpress MDM2 (Figure S1A, available online), and MDM2 is predominantly localized in the nucleus of cultured/untreated cells (Zhou et al., 2003). As is shown in Figure 1A, IR treatment decreased nuclear MDM2 and increased cytoplasmic MDM2 expression, and induced downregulation of MDM2 at serine 166 in EU-1 cells. IP western blot analysis demonstrated that MDM2 dissociated from p53 after IR treatment, as shown in Figure 1B, in which the levels of both MDM2 and p53 in immunocomplexed form precipitated with p53 and MDM2 antibodies, respectively, were significantly reduced in the IR-treated cells as compared with untreated cells.

We next evaluated whether there was concomitant upregulation of XIAP expression that would occur at a translational level, following the IR-induced cytoplasmic translocation of MDM2. We began by testing whether IR directly induces XIAP mRNA expression. Consistent with previous observation in other cancer cells (Holcik et al., 1999), XIAP mRNA was not induced by IR in EU-1 cell line (Figure 1C). The stability of XIAP mRNA was also not affected by IR treatment. As shown in Figure 1D, there was no difference in degradation rate of XIAP mRNA between IR-treated and untreated EU-1 cells. We next evaluated the effect of IR on XIAP translation in the MDM2-overexpressing cells. We treated EU-1 cells with IR in the presence or absence of the protein synthesis inhibitor cycloheximide (CHX). In the absence of CHX, IR induced XIAP, whereas XIAP was not induced by the same exposure of IR in the presence of CHX (Figure 1E). In order to directly demonstrate an increase in XIAP translation after IR in EU-1 cells, we further performed metabolic [^{35}S]-methionine labeling and IP analysis. As shown in Figure 1F, a significant increase in metabolically labeled, newly synthesized XIAP was apparent in the IR-treated cells, whereas the total XIAP protein levels were found to be equivalent in untreated versus IR-treated cells in the presence of the proteasome inhibitor MG132. In addition, we performed linear sucrose gradient fractionation to assess the polyribosome association of the XIAP mRNA in EU-1 cells subjected to IR and mock treatment. We found that XIAP mRNA was clearly shifted from fractions containing translation dormant complexes including mRNPs, ribosome subunits, and monosomes (Figure 1G, bottom, fractions 1–4) to fractions enriched of translating polyribosomes (Figure 1G, bottom, fractions 5–11), indicative of enhanced translation. Consistent with a previous observation (Lü et al., 2006), IR treatment had no effect on the polyribosome profile of actin mRNA (Figure 1G, top). Furthermore, there was no appreciable difference in XIAP protein stability between EU-1 cells exposed to IR and those without IR exposure (Figure 1H), thus demonstrating that induction of XIAP by IR occurs at the translational level.