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HMGA2 Inhibits Apoptosis through Interaction with ATR-CHK1 Signaling Complex in Human Cancer Cells[1,2]

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Abstract

The non-histone chromatin binding protein high mobility group AT-hook 2 (HMGA2) is expressed in stem cells and many cancer cells, including tumor initiating cells, but not translated in normal human somatic cells. The presence of HMGA2 is correlated with advanced neoplastic disease and poor prognosis for patients. We had previously demonstrated a role of HMGA2 in DNA repair pathways. In the present study, we employed different human tumor cell models with endogenous and exogenous expression of HMGA2 and show that upon DNA damage, the presence of HMGA2 caused an increased and sustained phosphorylation of the ataxia telangiectasia and Rad3-related kinase (ATR) and its downstream target checkpoint kinase 1 (CHK1). The presence of activated pCHK1^{Ser296} coincided with prolonged G_2/M block and increased tumor cell survival, which was enhanced further in the presence of HMGA2. Our study, thus, identifies a novel relationship between the ATR-CHK1 DNA damage response pathway and HMGA2, which may support the DNA repair function of HMGA2 in cancer cells. Furthermore, our data provide a rationale for the use of inhibitors to ATR or CHK1 and HMGA2 in the treatment of HMGA2-positive human cancer cells.

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Introduction

The small high mobility group AT-hook (HMGA) non-histone chromatin binding proteins HMGA1 and HMGA2 are composed of an acidic C-terminal tail and three separate N-terminal lysine– and arginine-rich AT-hook domains, which facilitate binding to the minor groove of short stretches of AT-rich DNA [1]. HMGA2 is expressed in embryonic stem (ES) cells, during embryogenesis, in some fetal tissues, and in some cancer cells. The protein is usually not detectable in normal adult somatic cells [2]. Phenotypically, HMGA1/2-positive cells display improved resistance to therapies that introduce chemical modifications of DNA bases, such as oxidation and alkylation [3–5]. *HMGA2* knockout mice exhibit a pygmy phenotype with greatly reduced fat tissues, and male mice are infertile [6,7]. By contrast, tissue-specific overexpression of full-length or

Abbreviations: ATM, ataxia telangiectasia mutated; ATR, ataxia telangiectasia and Rad3-related kinase; CHK, checkpoint kinase; CICs, cancer-initiating cells; DDR, DNA damage response; DNA-PKc, DNA-dependent protein kinase catalytic subunit; FLICA, Fluorescent Labeled Inhibitor of Caspases; HMG, high mobility group; HU, hydroxyurea; MMS, methyl methane sulfonate; RD, rhabdomyosarcoma

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ubiquitous expression of a truncated protein lacking the C-terminal tail results in gigantism, lipomatosis, and mesenchymal tumors [8,9].

We showed recently that HMGA2 remains associated with chromatin throughout the cell cycle in pluripotent human ES cells and that HMGA2 expression levels are further elevated during human embryoid body formation [10]. Furthermore, HMGA2 seems to be involved in the regulation of key human genes linked to mesenchymal cell lineage differentiation, adipogenesis, and human ES cell proliferation control [11]. We also demonstrated that HMGA1 and HMGA2 are linked to DNA base excision repair and this may have important implications for genome stability in ES cells and during early development and carcinogenesis [5].

Unique among DNA architectural chromatin binding factors, the *HMGA* genes are considered proto-oncogenes. HMGA1/2 proteins are consistently overexpressed in nearly all types of naturally occurring cancers and are important for multiple cellular processes including oncogenic transformation [12–15]. It has been recognized that high HMGA1/2 protein levels are associated with increased malignancy, metastatic potential, and poor clinical outcome [13,16–18]. HMGA2 expression is primarily regulated by the miRNAs *let-7* and *miRNA-98* during oncogenic transformation [19,20], but the molecular mechanisms linking *let-7* and HMGA2 with chemoresistance in cancer cells and cancer stem/initiating cells remain elusive [21].

Exposure of cells to DNA-damaging agents results in the activation of a signaling cascade aimed at arresting the cell cycle to repair the DNA damage or trigger apoptosis. The ataxia telangiectasia mutated (ATM) and ataxia telangiectasia and Rad3-related kinase (ATR) are related phosphatidylinositol 3-kinase-related kinases with important functions in the DNA damage response (DDR) pathways. ATM and its downstream target checkpoint kinase 2 (CHK2) constitute the main response to double-stranded DNA breakage [22]. The activation of the ATR and its downstream target CHK1 generally occurs in response to UV and agents that inhibit DNA replication forks [23–25]. ATR and CHK1 participate in the stabilization of forks, repair of DNA damage, and the inhibition of late origin firing [26–29]. The interaction between ATR and the ATR-interacting protein is essential for the phosphorylation of CHK1 and cells depleted of CHK1 accumulate multiple DNA breaks and undergo P53-independent apoptosis [30,31]. Recent evidence shows that the activated ATR-CHK1 pathway in response to fork inhibition preferentially inhibits the activation of new replication fork factories, defined as clusters of one or more adjacent replication origins [32–34]. This strategy conserves replication capacity for already active replicon clusters where forks are inhibited rather than engaging new replication factories, and this minimizes the risk of apoptosis [35]. Although different DNA-damaging agents can preferentially activate one of the two DDR signaling pathways [36], both ATM-CHK2– and ATR-CHK1–mediated DDRs are required for cell survival [31].

ATM was recently shown to interact with and phosphorylate HMGA2, and phosphorylated HMGA2 activated a positive feedback loop by upregulating ATM expression [37]. In the present study, we demonstrate a novel interaction between ATR-CHK1 and HMGA2 and provide evidence for a new cytoprotective role of HMGA2 by sustaining ATR-CHK1 phosphorylation. In four different cancer cell models used here, we show that the antiapoptotic activity of HMGA2 is mediated by activated pCHK1. Depletion of HMGA2, CHK1, or both factors resulted in mitotic cell cycle arrest, increased number of nuclear γ-H2AX foci, and caspase 3/7–mediated apoptosis with decreased resistance to the genotoxic agents methyl methane sulfonate (MMS) and hydroxyurea (HU). Thus, our data provide first evidence for an active role of HMGA2 in the ATR-CHK1 DDR pathway.

Materials and Methods

Cell Lines and Culture Conditions

We used established lung cancer A549-HMGA2 transfectants [5] and generated stable transfectants of the undifferentiated thyroid cancer cell line UTC8505 expressing human HMGA2 as described previously [38]. Transfectants were cultured in DME-F12 medium (Thermo Scientific, Ottawa, Ontario) plus 10% fetal calf serum (FCS; Sigma, Oakville, Ontario) and 500 μg/ml geneticin (Life Technologies, Burlington, Ontario). The fibrosarcoma cell line HT1080 (C1) harboring a doxycycline-inducible sh*HMGA2* construct was generated by standard lentiviral transduction using a *Tet-on* sh*HMGA2* construct, pTRIPz-sh*HMGA2* (Origene, Rockville, MD). Cells were grown in DME-F12 supplemented with 10% FCS and 3 μg/ml puromycin (Sigma). Induction of sh*HMGA2* was achieved with 4 μg/ml doxycycline (Sigma) for 48 hours with repeated doses every 24 hours and resulted in significant down-regulation of endogenous HMGA2. Human rhabdomyosarcoma (RD) cells were propagated in DME-F12/10% FCS. All cell lines were maintained in a humidified incubator under 5% CO_2 at 37°C.

Comet Assay

UTC8505 mock and HMGA2 transfectants (10^6 cells/ml) were cultured in serum-free DME-F12 medium and comet assays were performed as described earlier [5]. Images were obtained at 400-fold magnification using a chroma filter at 385-nm excitation/450-nm emission with a Z1 microscope (Zeiss, Jena, Germany). We used the (http://www.autocomet.com/products_cometscore.php) Comet Score Version 1.5 analysis software. DNA damage was quantified by the Olive tail moment [39].

Induction and Recovery Time Kinetic Assays

Cells (10^5 cells/well) in six-well plates were grown overnight. For induction time kinetics, DNA damage was induced by MMS treatment for 2, 5, 10, 15, 20, and 30 minutes and protein extracts were collected. For recovery time kinetics, cells were treated with MMS for 30 minutes and washed thoroughly twice with 1× phosphate-buffered saline (PBS) to remove MMS. Cells received fresh medium and were allowed to recover from MMS damage for 0, 1, 2, 4, 6, and 24 hours before total protein extraction.

Immunoblot Analysis

Proteins were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis and blotted onto nitrocellulose membrane (VWR, Mississauga, Ontario) followed by blocking with 5% skimmed milk in 1× Tris-buffered saline and 0.1% Tween 20 (pH 7.6). Membranes were incubated with the primary antibody overnight at 4°C in 5% BSA containing 1× Tris-buffered saline and 0.1% Tween 20 followed by the secondary antibody in blocking buffer for 1 hour at room temperature and detection of bands with the ECL Kit (Pierce, Nepean, Ontario). Primary rabbit polyclonal antibodies used were against phospho-ATR (pATR^{Ser428}), phospho-CHK1 (pCHK1^{Ser296}), phospho-CHK2 (pCHK2^{Thr68}), total ATR, total CHK1, and total CHK2