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Chk1 Suppresses a Caspase-2 Apoptotic Response to DNA Damage that Bypasses p53, Bcl-2, and Caspase-3

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SUMMARY

Evasion of DNA damage-induced cell death, via mutation of the *p53* tumor suppressor or overexpression of prosurvival Bcl-2 family proteins, is a key step toward malignant transformation and therapeutic resistance. We report that depletion or acute inhibition of checkpoint kinase 1 (Chk1) is sufficient to restore γ-radiation-induced apoptosis in *p53* mutant zebrafish embryos. Surprisingly, caspase-3 is not activated prior to DNA fragmentation, in contrast to classical intrinsic or extrinsic apoptosis. Rather, an alternative apoptotic program is engaged that cell autonomously requires *atm* (ataxia telangiectasia mutated), *atr* (ATM and Rad3-related) and *caspase-2,* and is not affected by *p53* loss or overexpression of *bcl-2/xl*. Similarly, Chk1 inhibitor-treated human tumor cells hyperactivate ATM, ATR, and caspase-2 after γ-radiation and trigger a caspase-2-dependent apoptotic program that bypasses p53 deficiency and excess Bcl-2. The evolutionarily conserved "Chk1-suppressed" pathway defines a novel apoptotic process, whose responsiveness to Chk1 inhibitors and insensitivity to *p53* and *BCL2* alterations have important implications for cancer therapy.

INTRODUCTION

The stress-inducible p53 protein acts as a central signal transduction node in the apoptotic response to DNA damage, mainly through its ability to transactivate intrinsic (mitochondrial) and extrinsic (death-receptor) pathway genes (Vousden and Lu, 2002). However, ample evidence supports the existence of p53-independent apoptotic responses to DNA damage. In *Drosophila* and mouse *p53* null embryos, for example, several cell types undergo apoptosis in response to irradiation (IR), but with slower kinetics than $p53^{+/+}$ cells (Frenkel et al., 1999; Wichmann et al., 2006).

Candidate p53-independent apoptotic pathways have surfaced from in vitro studies. ATM/ATR-activated ABL, Chk1, and Chk2 can upregulate p73 protein levels in genotoxically challenged p53-deficient cells, restoring transactivation of *PUMA* and other proapoptotic p53 targets (Gong et al., 1999; Roos and Kaina, 2006; Urist et al., 2004; Yuan et al., 1999). p53-independent coupling of DNA damage to mitochondria can also occur through translocation of the nuclear orphan protein Nur77 into the cytosol, activation of nuclear and/or cytosolic caspase-2, or de novo ceramide synthesis by mitochondrial ceramide synthase, all converging on caspase-3 activation (Kolesnick and Fuks, 2003; Li et al., 2000; Lin et al., 2004; Zhivotovsky and Orrenius, 2005). Other p53-independent processes, involving MAPKs (e.g., SAPK/JNKs, p38) and the transcription factors E2F1, NF-κB, and FOXO1 couple DNA damage to caspase-3 activation by upregulating extrinsic pathway genes including *CASP8*, whose product activates caspase-3 in a mitochondria-dependent (Bcl-2-inhibitable) or -independent manner (Afshar et al., 2006; Huang et al., 2006; Kasibhatla et al., 1998; Yount et al., 2001). Whether the p53-independent pathways identified in vitro operate in vivo remains an active field of investigation.

Radio/chemoresistant *p53* mutant human cancer cell lines can be induced to die after genotoxic stress by pharmacologic or RNAi targeting of DNA damage-response (DDR) kinases involved in intra-S and/or G2/M checkpoint control, including ATM, ATR, Chk1, Chk2, Polo-like kinases (Plks) (reviewed in Castedo et al., 2004a), and most recently, the p38/MAPK-activated kinase MAPKAPK2 (MK-2) (Reinhardt et al., 2007). Such treatments might spare cells endowed with wild-type p53, presumably because their intact G1 checkpoint enables them to repair and thus survive DNA damage (Zhou and Bartek, 2004). Although

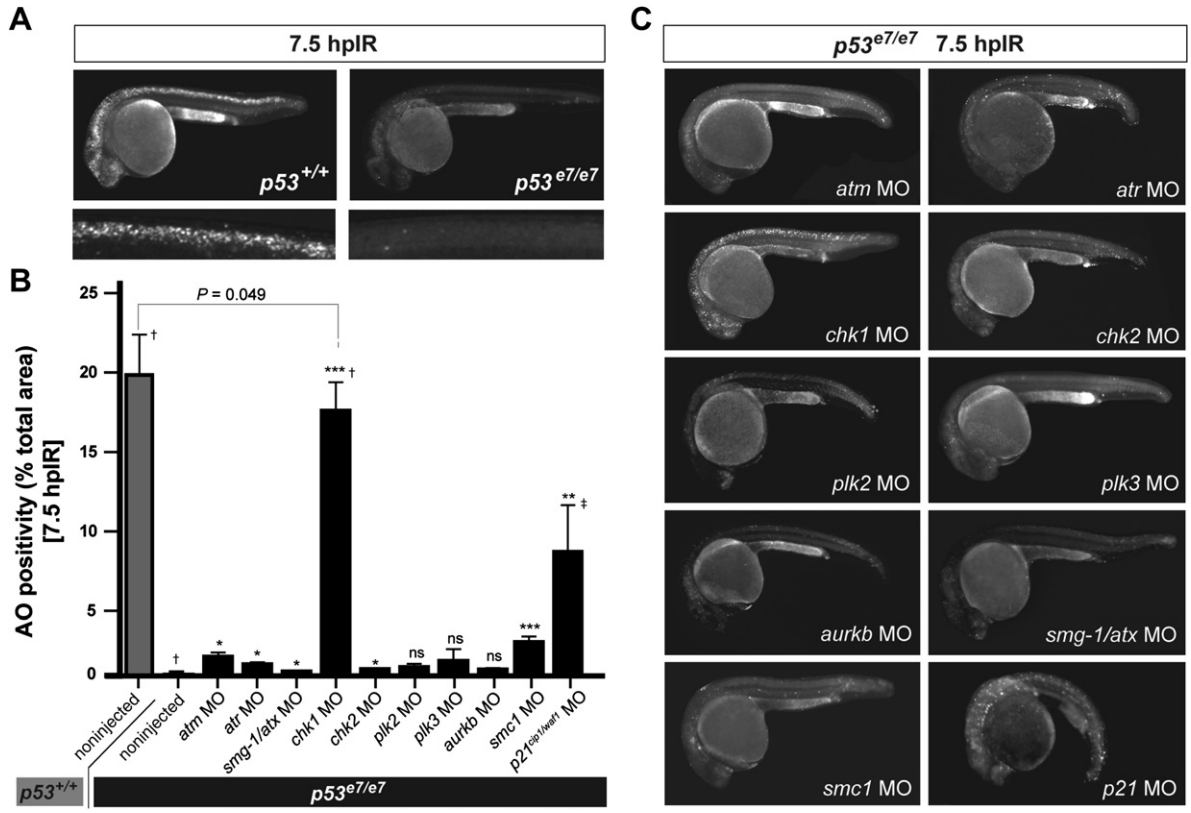

Figure 1. A Morpholino Screen Identifies *chk1* as a Loss-of-Function Suppressor of $p53^{e7/e7}$-Associated Radioresistance

(A) Live 25 hpf embryos of the indicated genotypes stained with AO at 7.5 hpIR (12.5 Gy). Anterior, left. Note the complete absence of AO labeling in the brain and spinal cord of the irradiated *p53* mutant.

(B) MO screen for loss-of-function suppressors of $p53^{e7/e7}$-associated radioresistance. Noninjected and 1 cell-stage MO-injected embryos were irradiated at 18 hpf (12.5 Gy). AO uptake by cells was quantified by analyzing images of whole embryos photographed live at 7.5 hpIR (y axis) (images as in C). Injected MOs are indicated along the x axis. Bars are color coded and refer to the genetic background used for injections (gray, $p53^{+/+}$; black, $p53^{e7/e7}$). AO staining was quantified in ≥8 embryos per knockdown, with 50 or more embryos scored per knockdown (except †> 1000); ‡, embryos showed developmental defects. All data are reported as means ± SEM. Statistical significance versus the noninjected $p53^{e7/e7}$ response: * $p < 0.05$; ** $p < 0.005$; *** $p < 0.0005$; ns (not significant), (two-tailed Student's t test).

(C) Fluorescent images of AO-labeled, live *p53* mutants injected with indicated MOs and representative of the phenotypes quantified in (B).

the sensitization of—and selectivity for—*p53* mutant cells is at the root of anticancer strategies that target DDR kinases, none of these concepts have been rigorously tested in an animal model, and the underlying cell death mechanism is unclear.

To accelerate the discovery of physiologic p53-independent DDRs, we generated *p53* mutant zebrafish lines for use in whole organism-based modifier genetic screens (Berghmans et al., 2005). Zebrafish faithfully recapitulate mammalian intrinsic and extrinsic apoptotic signaling (reviewed in Pyati et al., 2007). The zebrafish $p53^{M214K}$ allele (or $p53^{e7}$, for mutation in exon 7) affects a conserved amino acid residue within a region of the DNA-binding domain corresponding to a mutational hotspot in human cancer, producing a transactivation-dead p53 variant. Homozygosity for $p53^{e7}$ recapitulates key traits associated with p53 deficiency in mammalian systems, including a strong tumor-prone phenotype, lack of G1-checkpoint function, and widespread cellular radioresistance (Berghmans et al., 2005).

Here we identify *chk1* as a gene whose loss restores IR-induced apoptosis in live *p53* mutant zebrafish embryos, and then use in vivo epistasis analyses to dissect the underlying mechanism. Unlike previously identified p53-independent apoptotic pathways, which restore caspase-3 activation downstream of defective p53, Chk1 depletion activates an ATM/ATR-caspase-2 axis that bypasses the mitochondrial and death-receptor pathways. We show that this "Chk1-suppressed" pathway can be triggered in p53-deficient or *BCL2*-overexpressing human tumor cells, providing a mechanistic rationale for the use of Chk1 inhibitors in cancer therapy.

RESULTS

A Morpholino Screen for Suppressors of $p53^{e7/e7}$ Radioresistance Identifies *chk1*

$p53^{e7/e7}$ mutant zebrafish embryos are refractory to DNA damage-induced cell death, as demonstrated by a nearly complete lack of acridine orange (AO) labeling in the brain and spinal chord of live embryos examined 7.5 hr after whole-body IR delivered at 18 hr postfertilization (hpf) (Figures 1A and 2A). We used