DOCUMENT SUMMARY

This 2000 research paper by Ries et al. investigates the complex relationship between the **Ras** signaling pathway and the tumor suppressor protein **p53**. The study reveals that the Ras/Raf/MEK/MAP kinase pathway has opposing effects on p53: it directly activates the p53 inhibitor **Mdm2** (suppressing p53), while also inducing the Mdm2 inhibitor **p19ARF** (stabilizing p53). The balance between these two outputs determines the cell's fate, explaining how Ras-transformed cells can become resistant to DNA damage and apoptosis, a critical insight into cancer development and treatment resistance.

FILENAME

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METADATA

Category: RESEARCH **Type:** report **Relevance:** Core **Update Frequency:** Static **Tags:** #ras-pathway #p53 #mdm2 #p19arf #raf-mek-mapk #apoptosis #cancer-biology #signal-transduction #tumor-suppressor **Related Docs:**

- davis_2000_research_report_signal_transduction_jnk_mapk
- cheung_2000_research_report_histone_modification_epigenetics **Supersedes:** N/A

FORMATTED CONTENT

Opposing Effects of Ras on p53: Transcriptional Activation of mdm2 and Induction of p19ARF

Summary

Mdm2 acts as a major regulator of the tumor suppressor **p53** by targeting its destruction. Here, we show that the *mdm2* gene is also regulated by the **Ras-driven Raf/MEK/MAP kinase pathway**, in a p53-independent manner. Mdm2 induced by activated Raf degrades p53 in the absence of the Mdm2 inhibitor **p19ARF**. This regulatory pathway accounts for the observation that cells transformed by oncogenic Ras are more resistant to p53-dependent apoptosis following exposure to DNA damage. Activation of the Ras-induced Raf/MEK/MAP kinase may therefore play a key role in suppressing p53 during tumor development and treatment. In

primary cells, Raf also activates the Mdm2 inhibitor p19ARF. Levels of p53 are therefore determined by opposing effects of Raf-induced p19ARF and Mdm2.

Introduction

Mdm2 was originally identified as an amplified gene in a spontaneously transformed derivative of BALB/c cell line 3T3 DM, which caused tumors when injected into nude mice (Fakharzadeh et al., 1991). A possible mechanism for the transforming properties of *mdm2* has been provided by reports demonstrating that Mdm2 is a major regulator of the tumor suppressor **p53**. It binds directly to p53 and inhibits its transcriptional activity (Momand et al., 1992; Oliner et al., 1992, 1993). Mdm2 is a transcriptional target of p53 (Barak et al., 1993; Wu et al., 1993; Leng et al., 1995). Induction of *mdm2* transcription by p53 establishes a negative feedback loop, in which p53 itself initiates its own destruction (Picksley and Lane, 1993).

Mdm2 binds to **p19ARF** and is inhibited by this interaction (Kamijo et al., 1998; Pomerantz et al., 1998). An attractive model has been presented recently in which p19ARF binds to Mdm2, sequesters Mdm2 in nucleolar structures, and allows accumulation of p53 (Tao and Levine, 1999; Sherr and Weber, 2000).

The Ras/Raf/MEK/ERK kinase pathway has been reported to activate CDK4/cyclin D kinases, thereby phosphorylating pRb, which in turn leads to release of E2F-1 (Albanese et al., 1995; Peeper et al., 1997). The p14ARF promoter (human homolog of p19ARF) contains several E2F-1 binding sites. The Ras/Raf/MEK/MAP kinase pathway can therefore lead indirectly to accumulation of p14ARF and inhibition of Mdm2 activity.

Mdm2 expression is often increased following mitogenic activation. These data suggest regulation of *mdm2* expression by p53-independent pathways triggered by growth factors. A common feature of signaling by diverse growth factors is the activation of the Ras/Raf/MEK/MAP kinase pathway. We therefore explored the possibility that this pathway is responsible for *mdm2* induction.

Results

Mdm2 Expression Is Regulated by the Ras/Raf Pathway

To test whether the Ras pathway is responsible for *mdm2* induction, NIH-3T3 cells expressing an activated Ras allele, H-Ras (G12V), were analyzed. Expression of constitutively active Ras resulted in increased levels of *mdm2* mRNA and protein. We then examined the effects of a conditionally active Raf kinase. Addition of 4-HT (4-hydroxy-tamoxifen) to activate the kinase resulted in remarkable MAPK activation and accumulation of high levels of *mdm2* mRNA and protein. This induction occurred even in p53-deficient fibroblasts, confirming that the **Ras/Raf/MEK/MAP kinase pathway induces increased expression of *mdm2* mRNA and Mdm2 protein in a p53-independent manner.**

Analysis of Ras-Responsive Elements within the mdm2 P2 Promoter

Sequence analysis of the *mdm2* P2 promoter revealed the existence of binding sites for transcription factors of the **AP-1** and **Ets** family, which have been shown to be responsive to ERK activation in other genes. Using promoter deletion and mutagenesis experiments, we found that both an upstream Ets site and a downstream AP-1 site were critical for the Raf-induced transcriptional activation. Overexpression of known downstream targets of Ras and Raf (activated MEK1, c-Ets-1, c-Ets-2, and c-jun) also led to a 5- to 8-fold induction of *mdm2* P2 promoter activity.

Raf Regulates p53 Levels through Its Effects on Mdm2

Induction of Mdm2 protein expression is expected to decrease levels of p53. To test this, Raf kinase was activated in human tumor cells. In DKO4 cells (which lack p14ARF), activating Raf caused Mdm2 levels to accumulate. Importantly, **increased Raf activity led to a dramatic decrease of p53 protein levels**. In SW480 cells, which express an activated Ras allele, treatment with the MEK inhibitor U0126 resulted in a dose-dependent decrease in Mdm2 protein expression, showing that sustained activity of the pathway is necessary for high levels of Mdm2.

Ras Mutations Lead to Attenuation of the p53 Response and Increased Survival Rate upon γ-Irradiation

To investigate whether increased Mdm2 levels due to Ras activation can attenuate p53 induction in response to cellular stress, we γ-irradiated NIH-3T3 cells with and without activated Ras.

- In the parental NIH-3T3 cells, p53 protein was dramatically induced after γ-irradiation.
- In contrast, γ-irradiation of NIH-3T3 cells harboring an activated Ras led to only a moderate and delayed increase of p53 protein.

Thus, NIH-3T3 cells harboring an activated form of Ras are capable of attenuating the p53 response upon γ-irradiation, which is consistent with their increased basal Mdm2 protein expression.

This attenuation of p53 might explain the higher resistance of transformed cells to γ-irradiation. We confirmed this in human colon cancer cells (HCT116), where deleting the mutant Ras allele made the cells dramatically more sensitive to radiation. This effect was dependent on p53.

Attenuation of p53 Accumulation by Activated Raf Is Dependent on the p19ARF Status

p19ARF physically interacts with Mdm2 and consequently stabilizes p53. To investigate if p19ARF can prevent Raf-induced Mdm2 from degrading p53, we used wild-type and p19ARF-null mouse embryo fibroblasts (MEFs).

- In wild-type MEFs, activating Raf induced both Mdm2 and p19ARF. In these cells, the p53 response to DNA damage was **not** attenuated.
- In p19ARF-null MEFs, activating Raf induced Mdm2, which was then able to **attenuate** the p53 accumulation in response to DNA damage.

These results provide evidence that p19ARF is capable of neutralizing the increased Mdm2 protein in response to activation of the Ras/Raf/MEK/MAP kinase pathway. If p19ARF is not expressed... induced Mdm2 protein is fully functional, can bind to p53, and promote its degradation.

Discussion

In this study, we demonstrate that Mdm2 expression is also modulated by the Ras/Raf/MEK/MAP kinase pathway through activation of Ets and AP-1 sites in the P2 promoter, independent of p53 activity. Mdm2 induced by this pathway is functionally active and leads to the degradation of p53.

Importantly, the effects of induced Mdm2 on p53 are regulated by **p19ARF**. Ras therefore acts on p53 through two competing pathways. Activation of the Ras/Raf/MEK/MAP kinase cascade results in elevated levels of Mdm2 protein. However, in normal cells, this pathway also induces the expression of p19ARF, which inhibits Mdm2 activity.

> Thus, in normal cells, levels of p53 are determined by a balance between opposing effects of the Ras/Raf/MEK/MAP kinase pathway.

In many tumors, p53 function is abrogated by overexpression of Mdm2 or by loss of p14ARF (the human homolog of p19ARF). In about 30% of human tumors, Ras is activated by mutation. Ras-induced Mdm2 might block p53 from inducing apoptosis or growth arrest in the early phase of tumor development, allowing the coexistence of Ras mutations and wild-type p53.

In addition to a role in tumor development, Ras regulation of *mdm2* and p53 may have important implications in cancer treatment. Tumors carrying constitutively active forms of Ras might be more resistant to treatment with ionizing radiation and chemotherapy. We provide evidence that Mdm2 mediates the radioresistant phenotype conferred by oncogenic Ras.

In conclusion, we have shown that Mdm2 is a transcriptional target of the Ras/Raf/MEK/MAP kinase pathway, and that this activation is independent of p53. Ras therefore regulates p53 through opposing pathways involving Mdm2 and its inhibitor p14ARF. In cancer cells lacking p14ARF, Ras suppresses p53 expression. This may have important implications in cancer development and therapy.