Tumor Growth Suppression Through the Activation of p21, a Cyclin-Dependent Kinase Inhibitor

Nicholas Love
11/28/01

Introduction

A. What is p21?
- p21 is a gene found on chromosome 6 at 6p21.2
- this gene produces a protein involved in cell cycle regulation
- its protein function is to act as a cyclin-dependent kinase inhibitor (Cdk inhibitor) to promote cell cycle suppression through the inhibition of phosphorylation by cyclin-dependent kinases (Cdk’s)
**Introduction Cont’d**

B. How does p21 cause tumor suppression?

- tumor growth within tissues results from uncontrolled cell growth after DNA damage
- tumor suppression by p21 occurs in several steps:
  1. Cellular DNA becomes damaged by chemical mutagenesis or radiation
  2. Tumor suppressor gene, such as p53, is activated

---

**Introduction Cont’d**

3. p53 then binds to a response element on p21, which becomes activated
4. p21 then binds to either Cdk2, Cdk3, Cdk4, and Cdk6 to cause cell cycle arrest through its inhibition of cyclin-dependent kinases
5. Cell cycle arrest at Go/G1 continues until the DNA is repaired and p53 levels decline as a result of p21 inactivation
Structure and Function of p21

A. Function of p21
   1. Role of p21 in cell cycle regulation
      - acts as a Cdk inhibitor and promotes cell cycle arrest after DNA damage
   2. Location of p21 actions in cell cycle
      - the p21 protein product causes cell cycle arrest at G0/G1, which occurs just before S phases

Structure and Function of p21 Cont’d

3. Effectors of p21 include tumor suppressor genes and in particular p53, which binds to the response element on p21
4. p21 is able to bind to Cdk2, Cdk3, Cdk4, and Cdk6 at the restriction point, G0/G1, in the cell cycle
   - various concentrations of p21 are required for inhibition of different Cdk’s (See next slide for data results on inhibition by p21)
GST-Rb kinase assay using sf9 extracts and p21 concentrations

Structure and Function of p21 Cont’d

5. p21 deficiency has been shown to cause tumor growth in various tissues, including the pituitary and thyroid gland, liver, and pancreas.

6. The synergistic interaction of p21 and p18 can result in tissue specificity:
   - The loss of both p21 and p18 result in more rapidly forming tumors than p21 deficient mice, especially in the pituitary and lung.
   - No tumor growth is present when a functional Cdk inhibitor is present in the absence of p21, such as p18.
Tumor Formation in p21-/- mice occurs in various tissues

### P21 and Cyclin-Dependent Kinase Expression in Various Tissues

![Graph showing CDK6, CDK4, p27, p21, and p18 expression in various tissues]

**Fig. 3**: Expression of CDKs and CDK inhibitors in mouse tissues. Total cell lysates were prepared from the indicated wild-type tissues. Expression patterns of CDK6, CDK4, p27, p21, p18, and tubulin were determined by Western blot analysis. Tubulin expression was used to demonstrate equal loading of protein lysates.
Structure and Function of p21 Cont’d

B. Structure of p21

1. The promoter region and binding site for p53 can be shown through deletion analysis
   - deletions performed on the first 164 amino acid residues of the promoter
   - results indicate amino acid residues 1-80 are most important for inhibition of Cdk2 at the S phase restriction point

---

P21 Structure and Deletion Analysis

Figure 3. Deletion analysis of p21. The indicated C-terminal deletions of p21 were purified from E. coli as GST-fusion proteins and assayed for inhibition of Cdk2/cyclin A using histone H1 as substrate. Activities were quantitated by filter binding (solid symbol, 20 nM GST-fusion; open symbol, 200 nM GST-fusion).
Anti-Cancer Drugs and Their Interaction with p21

A. Daunomycin and its effects on p21 expression in HCT116 and MCF7 cells, which are human colorectal and breast cancer cells respectively

1. What is Daunomycin?
   - A drug which causes cell cycle arrest through the indirect activation of p21

Anti-Cancer Drugs and Their Interaction with p21 Cont’d

2. Western Blot Analysis Results
   - Increase in p21 expression with Daunomycin treatment in cancer cells

3. Analysis of p53 after Daunomycin treatment
   - Drug causes a greater amount of cells arrested at Go/G1 after treatment
   - Drug also causes an increase in cell apoptosis after treatment
Effects of Daunomycin on p21 expression in Human Cancer cells

Role of p53 in cell cycle arrest and apoptosis after daunomycin
3. Overall effects and benefits of Daunomycin treatment
   - Drug is a potent inducer of both p21 and p53 in human colorectal and breast cancer cells
   - Use of daunomycin allows inhibition of cellular metastasis through activation of p21

B. Effects of Apicidin on p21 activation in human prostate carcinoma cells
1. Apicidin
   - histone deacetylase inhibitor
   - activates expression of both p21 mRNA and protein (See next figure)
   - activation occurs through the accumulation of acetylated histones in the p21 chromatin
Induction of p21 mRNA and protein by Apicidin

![Image of Western blots showing induction of p21 mRNA and protein by Apicidin](image)

Anti-Cancer Drugs and Their Interaction with P21 Cont’d

2. Apicidin mechanism of action
   - Activates p21
   - Causes loss of viability in human prostate carcinoma cells in a concentration-dependent manner
Effects of apicidin on Human Prostate Carcinoma Cells

Anti-Cancer Drugs and Their Interaction with p21 Cont’d

3. p21 promoter activation by apicidin
   - Full-length vs. 93S p21 promoter luciferase reporter constructs (See next figure)
   - Results: 93S construct had higher amount of p21 induction
   - Conclusion: 93S section of p21 promoter is essential for p21 responsiveness to apicidin
P21 promoter activation by Apicidin

Anti-Cancer Drugs and Their Interactions with p21 Cont’d

4. Histone acetylation by apicidin
   - Levels of histone H3 and H4 acetylation increased over time in pancreatic carcinoma cells
   - Immunoglobulin control showed no histone acetylation (See next figure)
   - Conclusion: inhibiting histone deacetylase allows apicidin to promote hyperacetylation of specific histones, H3 and H4, in the chromatin of the p21 gene
H3 and H4 accumulation induction by Apicidin

Anti-Cancer Drugs and Their Interactions with p21 Cont’d

5. Overall effects and benefits of apicidin
   - Causes cell cycle arrest in pancreatic carcinoma cells through transcriptional activation of p21
   - Causes accumulation of acetylated histones H3 and H4 that are associated with the p21 promoter
   - p21 is a key target in growth inhibition by apicidin in several tissues besides pancreatic carcinoma cell lines
**P21 Transfection in Carcinoma Cell Lines**

A. Human Glioma Cells
   - Occur by a malignant transformation of low grade astrocytoma
B. Northern Blot analysis
   - Increase in p21 mRNA after p21 transfection into glioma cells (See next figure)

---

**Increase in p21 mRNA and Protein expression in p21 Transfectants**

p21 Transfection in Carcinoma Cell Lines Cont’d

C. p21 transfection vs. control cell lines
- p21 transfection promotes a decrease in tumor cell growth through the accumulation of p21 transfected cells in the Go/G1 phase (See next figure)

Effects of p21 transfection in Human Glioma Cells

Figure 2. Growth curve of U251MG human glioma cells. The growth of p21 transfectant was inhibited compared as control cells. The number of the cells at each point is the mean with standard deviation of four different wells. The asterisk represent significance at $p < 0.001$. 
D. Conclusions
- Decrease in glioma tumor cell growth by p21 transfection
- Growth inhibition was followed by Go/G1 cell cycle arrest as the result of p21 activation

Conclusions

A. p21 is directly activated by p53 binding to its response element in the promoter region
   - Occurs after DNA damage
B. Cells accumulate at the Go/G1 checkpoint
   - Occurs at this stage to prevent further S phase replication of the damaged cell
C. p21 binds to various cyclins in Go/G1, such as cyclin B, to promote Cdk inhibition and cell cycle arrest
Conclusions Cont’d

D. Apicidin and Daunomycin are currently used to treat patients that contain a p21 deficient gene

E. More current treatments also help to promote a reduction in tumor progression, including p21 transfection in cells containing a p21 gene deletion