p53 as Batman: Using a Movie Plot to Understand Control of the Cell Cycle

Instructor's Guide and Handout

Student Activity 1

Question 1. Which character in the Batman movie best represents p53? Justify your answer.

Batman: p53

[Batman] Protects Gotham: [cell] Guards the genome

Question 2. Which character best represents Mdm2? Justify your answer.

Joker: Mdm2

[Batman] Creates chaos by inactivating Batman: [cell] Inhibits p53

Question 3. Which character best represents ARF? Justify your answer.

Gordon: ARF

[Batman] helps Batman by stopping the Joker: [cell] Binds Mdm2, prevents it from binding p53

Question 4. Which molecules or cellular activities best represent the following elements in the Batman movie?

Two Face: Mdm2 Promoter

[Batman] A distraction that allows Joker to create more chaos: [cell] Overexpression of Mdm2 destabilizes p53, inhibiting p53 function

Batman's weapons: p21^{cip1} (cyclin-dependent kinase inhibitor)

[Batman] Batman's weapons that are needed to fight crime: [cell] p53 promotes the expression of p21^{cip1} to halt the cell cycle

Gotham: Genome

[Batman] The location that Batman and the Joker are fighting to control: [cell] The site where DNA damage occurs

Bat signal: ATM/ATR

[Batman] A way to alert Batman that he is needed: [cell] ATM/ATR are activated when DNA is damaged

Gotham police department: Endogenous cell defense

[Batman] A crime-fighting group in Gotham: [cell] Other mechanisms that the cell uses to protect itself

Lucius Fox: Chk2

[Batman] Provides Batman with his suit and activates his capabilities to stop crime: [cell] Chk2 is a kinase that phosphorylates and activates p53

Crime alert system: Cell cycle checkpoints

[Batman] A system that notifies Batman that crime is taking place in Gotham city: [cell] A mechanism for ensuring DNA is undamaged prior to cell division

Student Activity 2

Using the characters Batman, Lucius Fox, Gordon, Joker, and Two Face, answer how these events would affect the movie plot and action.

Scenario A: Commissioner Gordon arrests the Joker and brings him to jail.

Cell [Batman]: This represents a healthy cell [city], in which ARF [Gordon] binds to Mdm2 [Joker] and allows p53 to halt the cell cycle [solve crime.]

Scenario B: Detective Gordon is thought to be dead.

Cell [Batman]: This represents a cell with mutated ARF [Gordon], resulting in ARF deficiency. Loss of ARF function disrupts the balance between p53 [Batman] activation and inhibition, resulting in decrease in p53 [Batman] function and a rise in mutations, which can lead to cancer [crime].

Scenario C: Two Face joins the Joker.

Cell [Batman]: This represents a cell in which Mdm2 [Joker] is overexpressed due to a mutation in its promoter [Two Face] and thus the cell cannot initiate cell cycle arrest or apoptosis in response to DNA damage.

Session Two

Student Activity 3

How will the addition of an inhibitor of Mdm2 translation alter the following cellular properties? (see slide 72).

The abundance of Mdm2 protein (Figure 4A): The amount of Mdm2 will be close to zero for all cells.

The abundance of p53 protein (Figure 4B): The amount will be high in all cells.

The proportion of wild-type cells (Figure 4C) with cleaved caspase-3: The proportion will be high in all cells.

The proportion of mutant p53 cells (Figure 4D) with cleaved caspase-3 : The proportion will be high in all cells.

Student Activity 4

Convert the data in Post *et al*. (5) into a Batman movie plot. Small groups of students are assigned a figure to convert into a scene, then the class as a whole creates a logical series of related scenes that tell a story (see slides #81-82, for one possible outcome).

Rationale for the Batman plot shown in the slides based on the data in Post *et al*. (5):

Figure 1A: Mouse *mdm2* intron 1 containing the P2 promoter was replaced with human intron 1 with G or T polymorphism.

Figure 1B: Southern blot analysis of the DNA revealed correct targeting and single copy integration of both constructs at the *Mdm2* locus.

Figure 1 take-away message: Mdm2 alleles in mice were replaced with humanized alleles to create the mice with the altered genes (Mdm 2 SNP309T and SNP309G). *Mdm2* is now under the control of a mutant promoter.

[Batman scene]: Harvey Dent becomes the disfigured Two Face and teams up with the Joker.

Figure 2A: Mdm2 mRNA is more abundant in the Mdm2SNP309G/G mice than in the Mdm2SNP309T/T or C57Bl/6 mice.

Figure 2B: RNAse protection analysis for *mdm2* mRNA levels in spleens of Mdm2SNP309G/G and Mdm2SNP309T/T mice showed that *mdm2* was overexpressed from the P2 promoter in the Mdm2SNP309G/G. This confirms that inserting the G/G mutation in the P2 promoter works as expected.

Figure 2 take-away message: The expression of the *mdm2* gene is increased in the Mdm2SNP309G/G mice.

[Batman scene]: Encouraged by the presence of Two Face, Joker becomes much more active in Gotham.

Figure 3A: The expression of *mdm2* in mouse embryo fibroblasts from Mdm2SNP309G/G mice can be inhibited by a drug that blocks the activity of the transcription factor Sp1. This shows that Sp1 is the transcription factor acting at the site of the G/G polymorphism. This experiment measures mRNA abundance.

Figure 3B: The drug also decreases the abundance of the Mdm2 protein in the Mdm2SNP309G/G mice. This experiment measures protein abundance.

[Batman scene]: Poisoning Two Face decreases the activity of Joker.

Figure 3C: Mouse embryo fibroblasts from Mdm2SNP309G/G mice divide more rapidly than fibroblasts from Mdm2SNP309T/T mice. Because p53 inhibits proliferation, a rise in proliferation suggests p53 is not able to function properly. (No drugs were used here.) The potential for passing mutations on to new generations of cells increases.

Figure 3 take-away message: The increased abundance of the *mdm2* mRNA in the Mdm2SNP309G/G mice is due to increased gene expression caused by the mutant human *mdm2* promoter, not an increase in RNA stability. Mdm2SNP309G/G cells are more proliferative.

[Batman scene]: When Two Face and Joker team up, Joker becomes more active and more criminals come to Gotham, also raising the likelihood of crime. Poisoning Two Face can stop the increase in Joker's activity. The poison might also make some of the population in Gotham sick or die.

Figure 4A, B: Mdm2SNP309G/G mice exhibit increased Mdm2 and decreased p53.

[Batman scene]: Two Face distracts Batman from Joker, so he is not fighting crime or the Joker.

Figure 4C: Mdm2SNP309G/G mice exhibit less activation of apoptosis in response to DNA damage than do Mdm2SNP309T/T mice.

[Batman scene]: Apoptosis represents martial law imposed by the Gotham Police Department. Caspase is a police officer. When Joker is active and Batman is not, the effectiveness of the Gotham Police, as represented by Officer Caspase, is reduced, resulting in a crime spree.

Figure 4D: Mdm2SNP309G/G mice exhibit less activation of p53 downstream targets in response to DNA damage than do Mdm2SNP309T/T mice.

[Batman scene]: Puma and Ccng1 are represented as two officers of the Gotham Police force. Officer Puma is responsible for instituting martial law and Officer Ccng1 is responsible for imposing a curfew on the city. When Joker is active and Batman is not, Officers Puma and Ccng1 get knocked out and cannot impose martial law or a curfew.

Figure 4E: The effect of Mdm2SNP309G/G on radiation-induced caspase activity is similar when p53 is mutated (inactive).

[Batman scene]: When Batman is injured, the Two Face-Joker team promotes crime and suppresses police effectiveness.

Figure 4 take-away message: There is an inverse correlation between the abundance of Mdm2 and the abundance of p53 and between Mdm2 abundance and the ability to activate cell cycle arrest and cell death pathways in response to DNA damage.

[Batman scene]: When Two Face is in Gotham, he and Joker join forces, creating a large criminal network that Batman cannot effectively contain.

Figure 5A, B: The reduced apoptosis caused by overexpressing Mdm2 increases the likelihood the mice will develop cancer. Mdm2SNP309G/G mice exhibit reduced survival compared to Mdm2SNP309T/T mice. This is exacerbated in mice with a mutant *p53* allele.

[Batman scene]: An influx of criminals occurs when Two Face and the Joker have joined forces, Gotham experiences destruction and damage, which is worse when Batman is injured.

Figure 5 C: Tumors in the Mdm2SNP309G/G mice were more frequently positive for Mdm2 and had less active caspase than did tumors in the Mdm2SNP309T/T mice.

Figure 5D: Loss of heterozygosity in mice expressing one mutant allele of *p53* did not differ in Mdm2SNP309G/G versus Mdm2SNP309T/T mice.

Figure 5 take-away message: The long-term consequences of expressing the Mdm2SNP309G/G allele are (i) a higher risk of developing cancer, (ii) a quicker onset of cancer, and (iii) a reduced ability to stop cancer by inducing apoptosis.

[Batman scene]: When Two Face and Joker join forces and throw the city into turmoil, the people in Gotham city are struck by panic and fear. The people yield to Joker's demands and threats, which leads to more chaos and crime and damage to the city. This overwhelms Batman's ability to handle so many simultaneous crises, and impairs his ability to work with the police. Batman fails to be the hero that the city desperately needs.

Figure 6 A-K: Mdm2SNP309G/G mice develop different types of tumors than Mdm2SNP309T/T mice and these tumors are similar to those found in humans with this mutation in *MDM2*.

Figure 6 take-away message: SNP309G/G may contribute to some forms of cancer in humans, especially breast cancer.

[Batman scene]: Without Batman protecting the city, additional criminal groups come to Gotham and terrorize the people of Gotham.

Summary of Batman Scenes, per the Post *et al*. paper:

Harvey Dent becomes the disfigured (mutant) Two Face and turns evil, much as he did in The Dark Knight. He decides to join forces with Joker. Scenes in this script show Two Face directly triggering Joker's activity in Gotham, including suppressing Batman's crime-fighting ability. As a result, the city becomes more crime ridden, such that even the declaration of martial law is only partially effective in reducing crime. Several members of the Gotham police force are incapacitated when Batman falls victim to Joker, and the situation only worsens when Batman is injured. The resulting chaos leads to catastrophic damage and loss in the city.

Possible discussion questions:

1. Why didn't Batman (p53) use his weapons (for example, p21^{cip1}) in the Post paper? What effect might they have had? What other weapons does he have available (such as other CDK inhibitors, inhibitory kinases that target CDK, or kinases that phosphorylate and activate p53)

2. How could have Commissioner Gordon (ARF) have helped overcome the Two Face-Joker team?

[Cell] Sequester Mdm2: [Batman] Put Joker in jail

3. What kind of characters would represent ATM and ATR?

ATM and ATR could be cops on the beat, arresting Joker when they spot him.

How are these different from Commissioner Gordon (ARF)?

[Cell] ARF sequesters Mdm2 directly, whereas ATM/ATR only modify Mdm2: [Batman] Commissioner Gordon puts Joker in jail; cops just chase the Joker

4. Would the poison that inhibited Sp1 and stopped Mdm2 (Two Face) from functioning be a possible treatment for cancer?

In instances where the Sp1 transcription factor is causative for cancer, mithramycin A is an effective treatment. These Sp1-dependent cancers include myeloid cell leukemia and non-small cell lung cancer. Because Sp1 functions in a number of different tissue types, specificity for tumor cells can be a problem.

The Batman Sessions

Handout 1

Activity 1: Using the information presented so far in class, plus your assigned reading, which character in the Batman movie best represents p53? Justify your answer.

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Which character best represents Mdm2? Justify your answer.

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Which character best represents ARF? Justify your answer.

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Activity 2: Which molecules or cellular activities best represent the following elements in the Batman movie? Include some rationale for your choices.

Two Face:

Batman's weapons:

Gotham:

Bat signal:

Gotham Police Department:

Lucius Fox:

Crime alert system: