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Regulation of the MDM2-p53 Nexus by a Nuclear Phosphoinositide and Small Heat Shock Protein Complex

by

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CHAPTER FOUR: WISCONSIN INITIATIVE FOR SCIENCE LITERACY

This chapter was designed to describe this study to non-scientists, and is sponsored by the Wisconsin Initiative for Science Literacy, it was written by Jeong Hyo Lee with support from Elizabeth Reynolds, Bassam Z. Shakhashiri and Cayce Osborne.

Figure 4.1 by Jeong Hyo Lee

Figure 4.2 by Jeong Hyo Lee

Figure 4.3 by Jeong Hyo Lee

Figure 4.4 by Jeong Hyo Lee

A Hidden Messages from the Inside: How Lipids Shape the Cell's Fate

Prelude

"The course of true love never did run smooth."

- *A Midsummer Night's Dream*, William Shakespeare

After taking my first undergraduate biology lab course, I became fascinated by how experiments could trigger reactions in tiny organisms. One technique, called Polymerase Chain Reaction (PCR), especially caught my interest. PCR allows scientists to copy specific parts of DNA, and it uses an enzyme originally found in a bacterium called *Thermus aquaticus*. To see where this discovery was made, I spent all my savings to visit Yellowstone National Park, where *T. aquaticus* was first found. That trip was more than a visit. It inspired me to dedicate my life to scientific discovery. I fell in love with research.

But as the line from the play *A Midsummer Night's Dream*, "The course of true love never did run smooth." Research has been challenging. Still, I believe effort and dedication can lead to powerful discoveries. Through my academic journey, I've learned to be patient and persistent. I'm committed to asking questions, finding answers, and pushing forward. My passion for research hasn't faded, it continues to grow. Through this chapter, I hope my passion will inspire others to begin their own research journeys, just as I did.

Introduction

Every second, trillions of decisions unfold silently inside your body, especially in the cells: cells must decide when to divide, when to repair, when to rest. These decisions rely on complex communication networks. For years, scientists have worked to explain how these communications work, what happens when cell communication systems fail, and how we can fix those failures. My graduate school research adds a new piece to the puzzle.

This chapter explains a major discovery: that certain lipids could provide a new perspective on cell communication in the nucleus. “Lipids” are a group of organic compounds that includes fats, waxes, and some vitamins (such as A, D, E, and K). While lipids’ most commonly known function is to store energy as fat, lipids also serve as structural components of cell membranes. Another class of lipids is phosphoinositides; these lipids serve as signal messengers inside cell membranes. Our laboratory’s main focus is investigating how phosphoinositide regulates other proteins in the cell nucleus. Among these phosphoinositide lipids is PIP_2, which works inside the cell’s nucleus to control cellular communication.

Since I was interested in cancer biology, I began my Ph.D. research on PIP_2 because, prior to me joining the lab, a former member had discovered that PIP_2 in the cell nucleus regulates the cancer-related protein p53. This hidden layer of communication plays a surprising role in regulating various important proteins related to cancer development. By showing PIP_2 helps control these specific proteins, my research opens the door to new strategies for treating diseases, especially cancer. Let’s start by understanding the key players.

Meet the Cast: Proteins and Lipids that Run the Cell

Cells don't have brains, but they still make decisions. To do that, they rely on proteins, large, complex molecules that perform tasks and relay information. Among them, few are more famous than p53. The guardian of the genome, p53 is a protein that protects the cell's DNA from numerous stresses. It helps repair damage and, if necessary, can stop the cell from dividing or even trigger cell death. This is especially important in preventing cancer. If a cell becomes abnormal, p53 acts like a security guard, shutting things down before harm spreads. As mentioned above, one of our laboratory members discovered that PIP_2 helps maintain p53 levels in the cells.

To reduce p53, the cell uses another protein called MDM2, which is known as the master of guardians. How fascinating! Not just a guardian, it is the master of guardians. I was very interested in this cool-nicknamed protein. When cells need to eliminate a specific protein, they call the cell's janitors, the proteins E1, E2, and E3. These proteins work together to mark certain proteins as trash by using a chemical tag, ubiquitin, like "OK to Trash" stickers. Ubiquitin-marked proteins are degraded by the proteasome, the cell's recycling bin.

MDM2 binds to p53 and marks p53 (by using ubiquitin) for destruction when it's not needed. This balancing act, between protection and restraint, keeps the cell healthy. But like any balancing act, it's a classic love-hate relationship. When p53 levels rise, MDM2 is produced. This increase in MDM2 then leads to a reduction in p53 levels. Understanding the interaction between p53 and MDM2 is critical for cancer research (Figure 4.1).

Now meet PIP_2. This molecule is a phosphoinositide, a type of lipid. It's part of the cell's inner membrane and has long been known to send signals from the cell's surface inward. Researchers have recently uncovered where PIP_2 shows up and what it does within the cell. Researchers have found PIP_2 deep inside the cell nucleus, bound directly to proteins like p53, and MDM2. That discovery changes how we think about communication inside the cell.

Lipid Signaling in the Nucleus: A New Kind of Messenger

To understand my research, it helps to think about how messages normally move through the cell. Inside the cell, there are first, second, and third messengers. When a hormone or other signal hits the outside of a cell, it starts a chain reaction. The signal is picked up by a "receptor" on the membrane, like someone answering a phone. That's the first messenger. The receptor then activates smaller molecules inside the cell, called second messengers. These travel through the cell and activate other proteins. It's like a relay race: signal in, response out. But until recently, scientists thought this race stopped at the edge of the nucleus. However, my new research suggests there exists a third messenger, PIP_2, which continues the communication inside the nucleus (Figure 4.2). It binds directly to nuclear proteins and changes their behavior. This concept of a third messenger within the cell is entirely new.

The Discovery: How PIP_2 Shapes the MDM2-p53 Nexus

My study explored how nuclear PIP_2 influences the stability and activity of MDM2 and p53[2]. The first thing we have to ask: does MDM2 interact with PIP_2? I performed a series of

experiments that mixed MDM2 with different artificial, laboratory-produced lipids, then evaluated their binding with MDM2. I found that MDM2 strongly preferred binding to PIP_2 over other similar lipids. This suggests that PIP_2 has a specific role, it isn't just sticking to proteins at random.

Next, I looked inside living cells. I used a technique called metabolic labeling, which marks phospholipid and tracks newly made PIP_2 in the cells. This test revealed that newly made PIP_2 is linked to MDM2, especially when the cells were under stress (Figure 4.3). Additionally, I measured the intimacy (that is, proximity) between MDM2 and PIP_2 in the nucleus of living cells with high-resolution imaging, using a technique called a proximity ligase assay. This technique was invented by one of our previous laboratory members. When I used a drug to induce DNA damage in my sample cells, I found that MDM2 measures in close proximity with PIP_2 most prominently in the nucleus (Figure 4.4). That's important. It means that the association between MDM2 and PIP_2 isn't just test-tube chemistry, it happens in real cells, and it responds to changes in the environment.

Where does this nuclear PIP_2 come from? I mentioned above that PIP_2 is one of the phosphoinositides, or more simply: a signaling lipid within our cells. A single phosphoinositide can transform into a different type of phosphoinositide, but this process needs a specific protein to induce the transformation. The protein, PIPKIα, can convert different phospholipid into PIP_2. According to my results, PIPKIα interacts with MDM2 in the nucleus and facilitates the binding of MDM2 to PIP_2. When cells were stressed, more PIP_2 appeared near MDM2, leading to increased binding between MDM2 and PIP_2. When I used a method called RNA interference to reduce PIPKIα levels, levels of PIP_2 also decreased, and so did MDM2 levels. This suggested that PIPKIα

is needed to stabilize MDM2 by generating PIP_2 right where it's needed. In other words, PIP_2 isn't just a floating molecule. It's made on-site, at the exact spot where it's used. It's like a coffee machine placed in your office instead of the kitchen down the hall. You need the coffee right at your desk to keep working. PIPKIα acts like that machine, brewing PIP_2 exactly where MDM2 needs it to stay functional. That kind of precision is a hallmark of tightly controlled systems.

Now, I introduce two more players: small heat shock proteins (sHSPs), specifically HSP27 and αB-crystallin (αBC). Heat shock proteins are so named because they are produced when cells are exposed to elevated temperatures; heat shock proteins work to protect cells from high heat. Other forms of stress also enhance heat shock proteins' production. Among this class of heat shock proteins, there are small proteins, known collectively as small heat shock proteins. These proteins act like chaperones at a fancy party. They help escort and stabilize other proteins, especially under stress.

I discovered that PIP_2 changes which sHSP chaperone binds to MDM2. Without PIP_2, MDM2 mostly binds to HSP27. With PIP_2, MDM2 shifts to bind more strongly to αBC. This made me curious. Why were they showing different effects? What I found was intriguing. These two chaperones have opposite effects: αBC stabilizes MDM2 and helps it interact more with p53, while HSP27 reduces MDM2's ability to bind p53. In this way, PIP_2 acts like a doorman choosing which chaperone gets in the room with MDM2.

Functional Consequences: More Than Just Binding

All these molecular handshakes affect real outcomes in the cell. When PIP_2 is added to the mix, MDM2 binds to p53 more tightly, up to a point. This effect works even when p53 itself can't bind PIP_2, suggesting that the PIP_2-MDM2 bond is the key driver. This matters because tighter binding between MDM2 and p53 is a prerequisite for p53 degradation. But the chaperone effect complicates things.

My study showed that αBC stabilizes MDM2 by preventing it from being degraded in the cell's "recycling bin," the proteasome. PIP_2 helps αBC bind MDM2, strengthening this protective effect. On the other hand, HSP27 leads to more MDM2 degradation. This part was particularly challenging for me. In the case of p53, PIP_2 recruits both HSP27 and αBC, and both contribute to stabilizing p53. This apparent inconsistency kept me up at night. One day, my daughter Gia was playing with a remote control. Watching her switch channels inspired a model explaining how PIP_2 differentially regulates the interaction between MDM2 and small heat shock proteins, and how these differences ultimately determine the cell's fate. I might give her an authorship for this idea. Just kidding! Anyway, PIP_2 acts as a remote controller, turning on stabilization when it brings in αBC and turning it off when HSP27 dominates (Figure 4.5). I also found that PIP_2 affects how MDM2 adds chemical tags to itself and to p53. This tagging marks proteins for destruction. By shifting who binds to MDM2, PIP_2 influences who gets tagged, and who survives.

Broader Implications: New Pathways, New Treatments?

My research reshapes how we think about nuclear phosphoinositides and their role in disease. The idea that lipids can act inside the nucleus, not just the membrane, is still new. I propose

that PIP_2 may be part of a larger class of "third messengers" that directly link to key proteins and change their behavior at the gene-control level. If true, this could explain previously mysterious aspects of gene regulation and stress responses.

Since MDM2 and p53 are central to cancer, any discovery that changes our understanding of their regulation could lead to new therapies. For example: Drugs that mimic or block PIP_2 in the nucleus could control how much p53 is active. Therapies that shift the balance of αBC and HSP27 could fine-tune this regulation. Targeting these mechanisms could allow treatments that are more specific, avoiding the widespread effects of current cancer drugs.

The study ends by raising important new questions: How many other proteins in the nucleus are associated with PIP_2? Are similar lipid-based "linkages" regulating other signaling pathways? Can these effects be manipulated safely in living organisms? I want to answer all of these questions, but if I tried, I would never leave the laboratory. So, I will leave them to future members of the laboratory. Answering these will take time, and likely require more tools, including advanced imaging, new labeling methods, and in vivo models. But two things are clear: nuclear lipids are not passive bystanders, but instead are active, selective, and powerful regulators of cell fate. And solving these questions will be FUN!

Conclusion: A Hidden Layer of Control

My research reveals a hidden layer of cellular control, one that operates through molecules long overlooked in the nucleus. By showing how PIP_2 links to and regulates MDM2 and p53, and

by identifying the role of chaperone proteins, αBC and HSP27, in this process, the study opens a new chapter in our understanding of cell signaling. It challenges textbook views and offers a new framework for exploring diseases like cancer. What once seemed like a simple lipid molecule turns out to be a master remote controller, helping decide whether a cell lives, dies, or divides. That's not just surprising. It's a fundamental shift in how we understand life at the molecular level.

Figures for Chapter Four

Figure 4.1

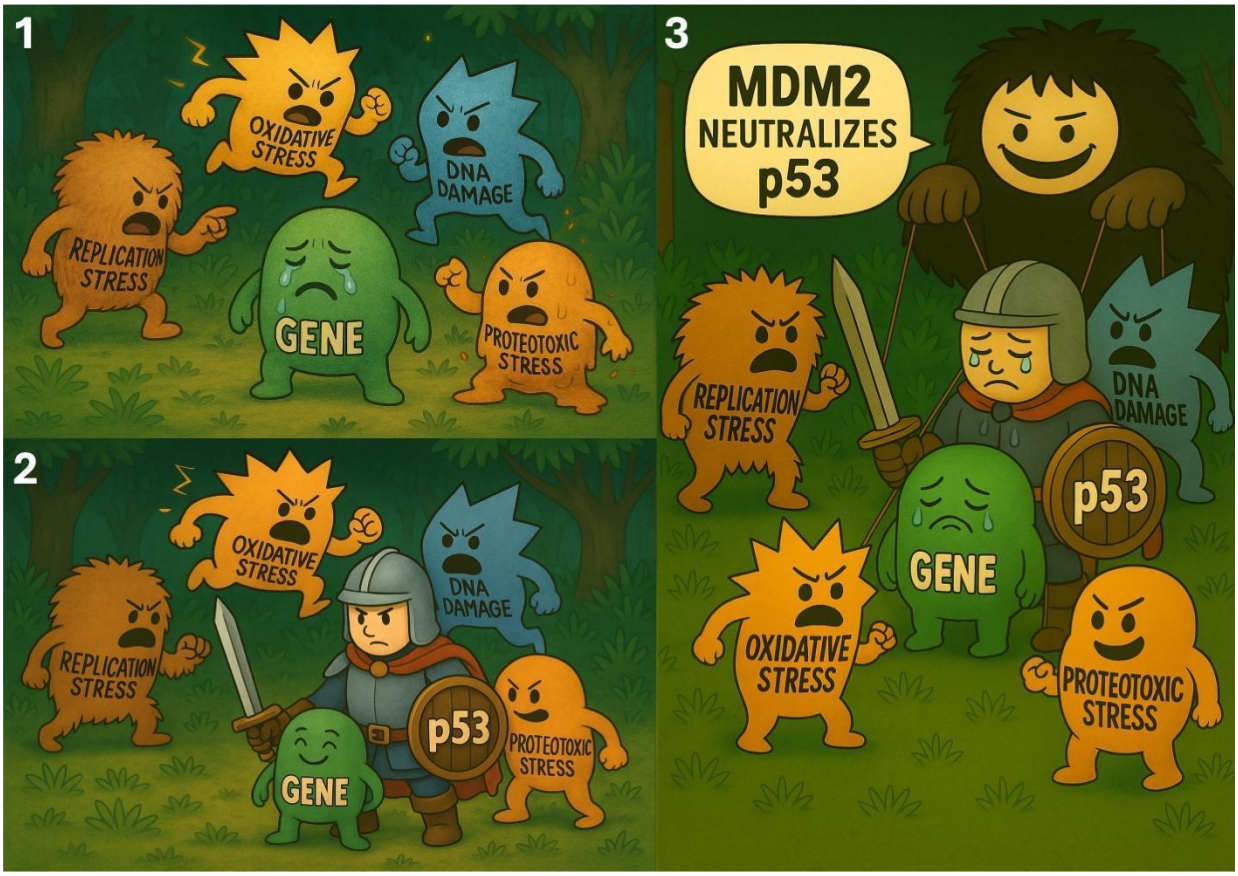


Figure 4.2

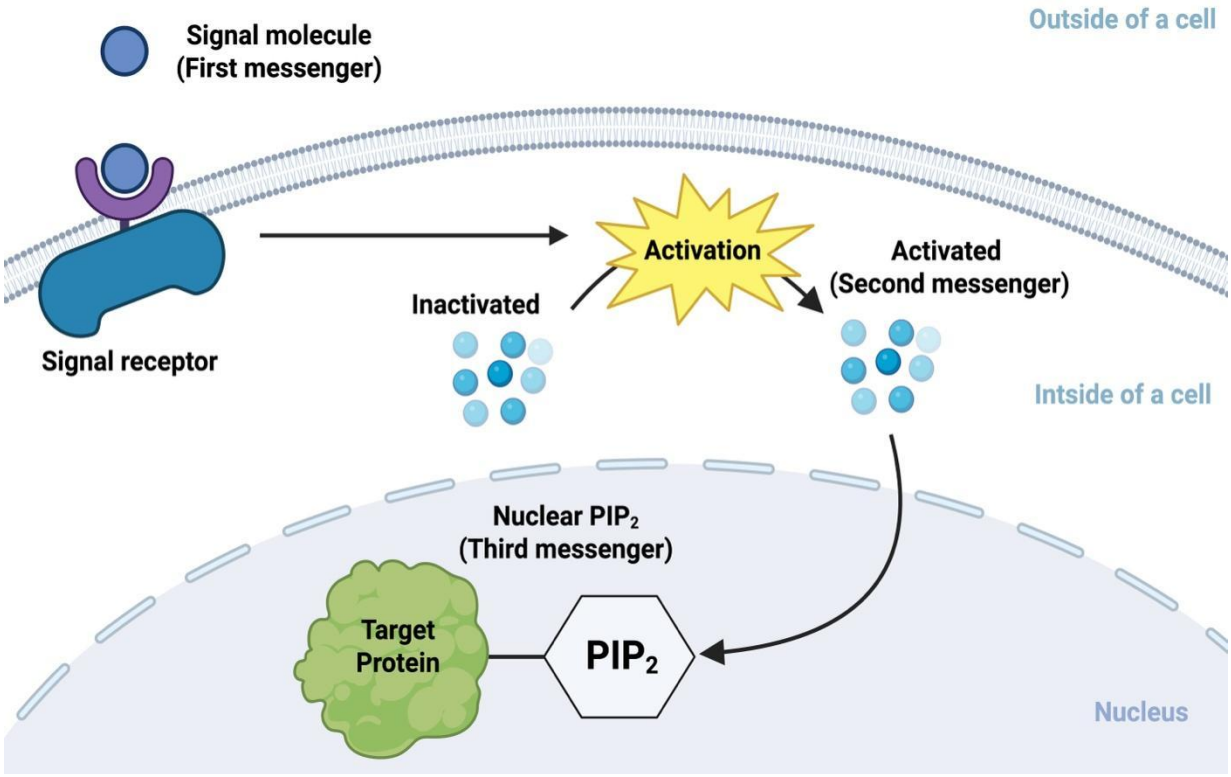

Signal molecule
(First messenger)
Outside of a cell
Signal receptor
Activation
Inactivated
Activated
(Second messenger)
Intside of a cell
Nuclear PIP2
(Third messenger)
Target
Protein
PIP2
Nucleus

Figure 4.3

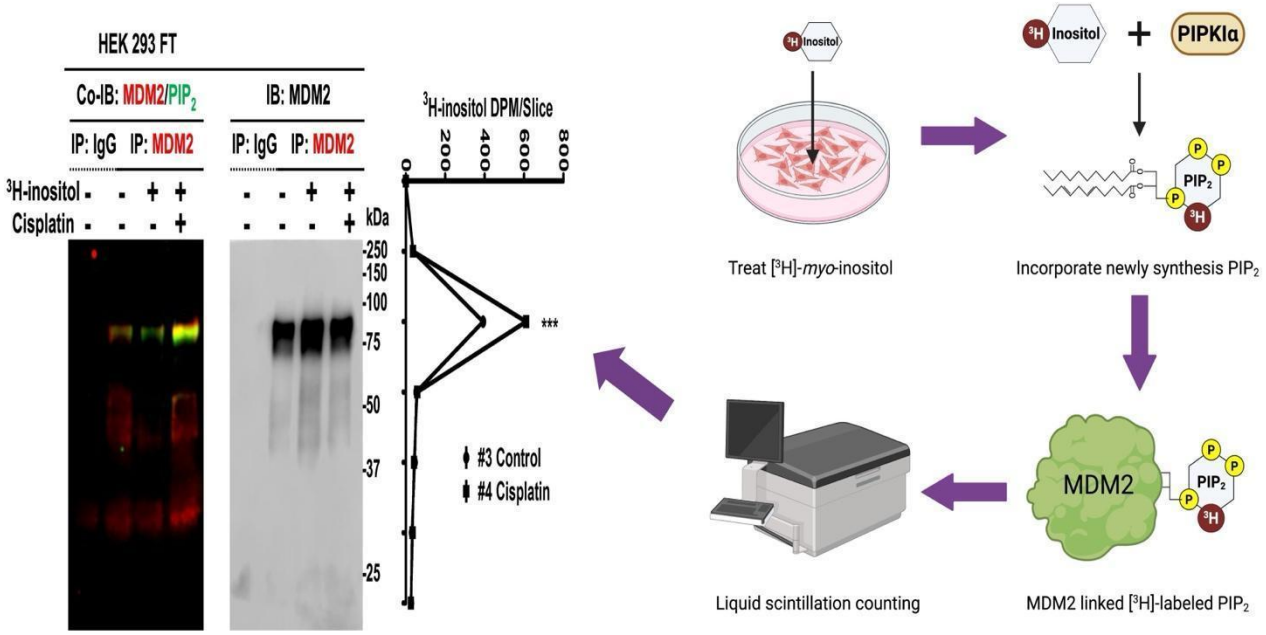

HEK 293 FT
Co-IB: MDM2/PIP2
IB: MDM2
IP: IgG IP: MDM2
IP: IgG IP: MDM2
3H-inositol
Cisplatin
kDa
-250
-150
-100
-75
-50
-37
-25
3H-inositol DPM/Slice
0
200
400
600
800
***
#3 Control
#4 Cisplatin
3H Inositol
Treat [3H]-myo-inositol
3H Inositol + PIPKIα
PIP2
Incorporate newly synthesis PIP2
MDM2
MDM2 linked [3H]-labeled PIP2
Liquid scintillation counting

Figure 4.4

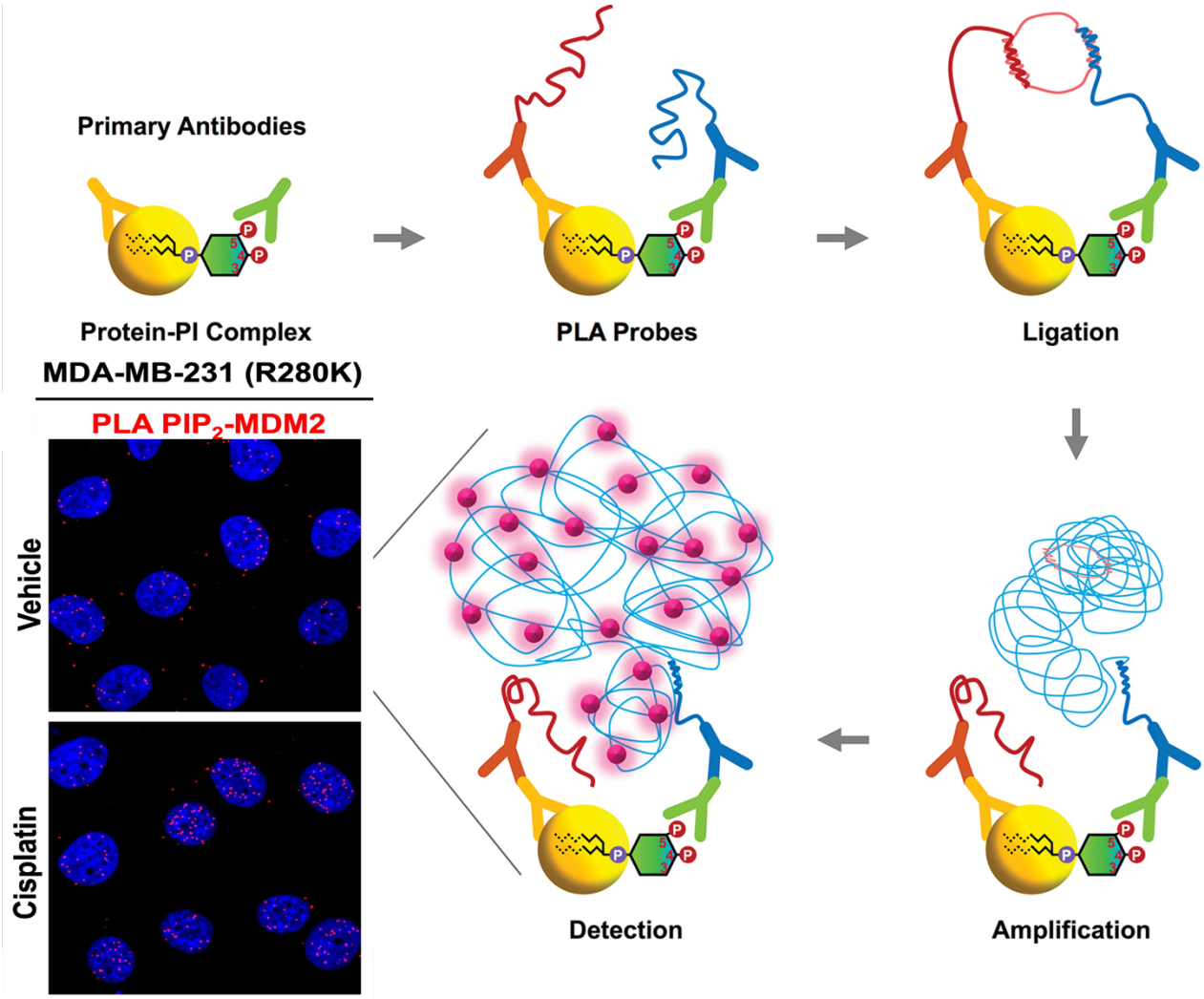
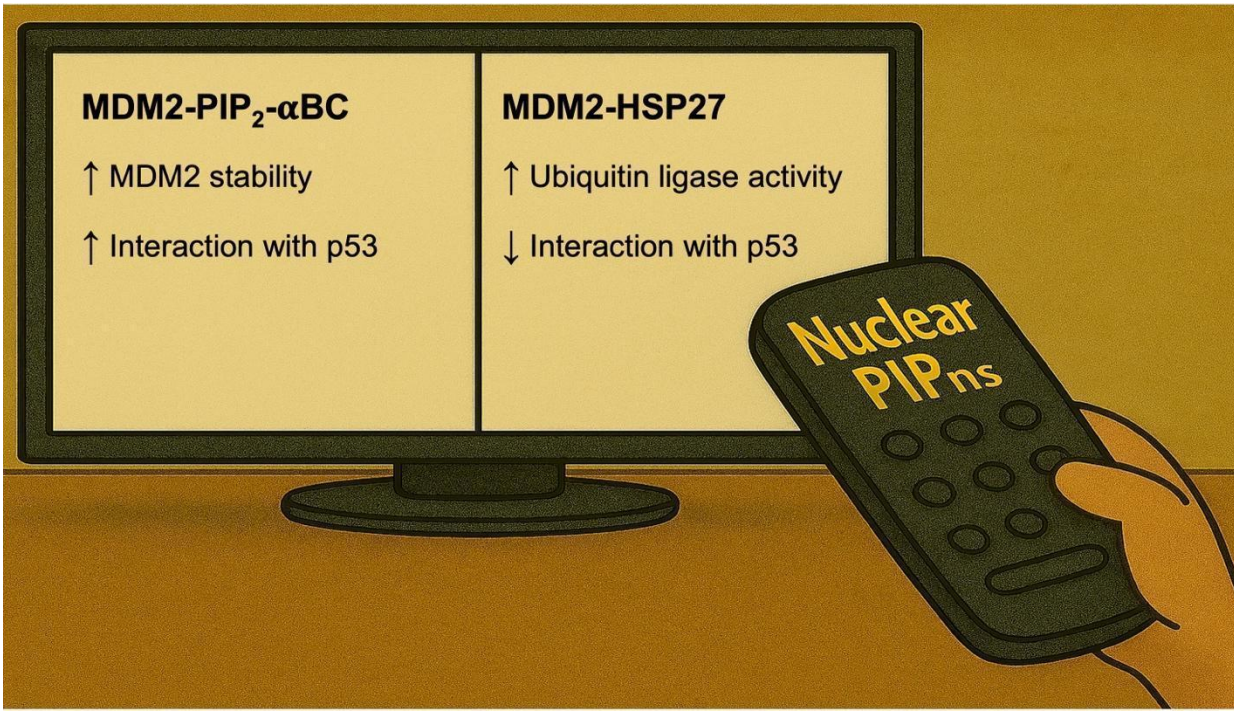

Primary Antibodies
Protein-PI Complex
PLA Probes
Ligation
MDA-MB-231 (R280K)
PLA PIP2-MDM2
Vehicle
Cisplatin
Detection
Amplification

Figure 4.5


MDM2-PIP2-αBC
↑ MDM2 stability
↑ Interaction with p53
MDM2-HSP27
↑ Ubiquitin ligase activity
↓ Interaction with p53
Nuclear PIPns

Figures Legend

Figure 4.1 The guardian of genome, p53 and the master of guardian, MDM2

Numerous stresses such as oxidative stress, DNA damage, replication stress, and proteotoxic stress could mutate genes and induce cancer. The guardian of the genome, p53, protects genes from these stresses. Additionally, p53 starts cell division and repair processes, and when cells are damaged, it initiates the cell death process to prevent normal cells from turning into cancer cells. The master of guardians, MDM2, binds to p53, blocking p53 function and tagging p53 for degradation by using a specialized chemical tag called ubiquitin.

Figure 4.2 A schematic model of the three different messenger systems

Each messenger works in a different location in the cell. The first messengers are hormones or other signals from outside of the cells that bind to receptors on the membrane. The second messengers are activated smaller signal molecules inside of the cells. Finally, the third messengers are tightly linked to target proteins to regulate their stability and activity in the nucleus.

Figure 4.3 A model of metabolic labeling to test linkage between MDM2 and PIP_2

First, radiolabeled phospholipids were treated into the live cell culture. Then radiolabeled phospholipids interconvert into PIP_2 and linked to MDM2 by PIPKIα. By using a liquid scintillation counter, we assess how much radiolabeled PIP_2 is present in the samples.

Figure 4.4 A model of proximity ligase assay to test proximity between MDM2 and PIP_2

First, two primary antibodies recognize the MDM2-PIP_2 complex in the cell. Then secondary antibodies coupled with probes bind to the primary antibodies. Next, the probe from MDM2 and probe from PIP_2 interact when they are in close proximity. Through the amplification process, we could detect signals in the cells.

Figure 4.5 A cartoon showing how nuclear PIP_2 selectively regulate MDM2-sHSPs

Nuclear PIP_2 acts like a remote controller to decide which small heat shock proteins interact with MDM2. In the presence of PIP_2, it helps αBC bind MDM2 to stabilize and increase interaction of MDM2-p53, leading to p53 degradation. On the other hand, in the absence of PIP_2, HSP27 directly interacts with MDM2 and decreases binding of MDM2-p53. So again, PIP_2 acts as a remote controller, to decide the fate of the MDM2-p53 nexus.