PPAR-γ and DNA Damage in Pulmonary Arterial Hypertension

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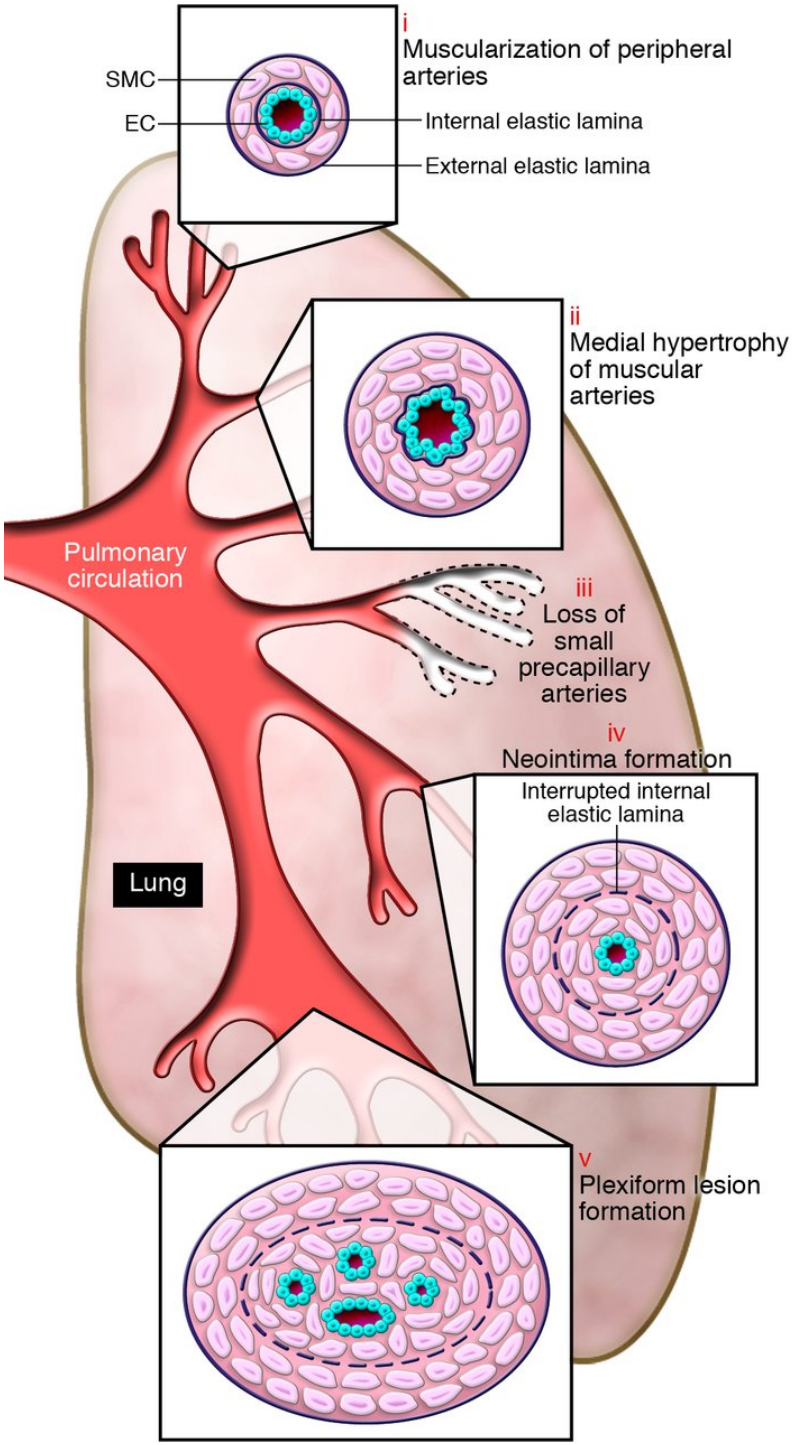
Pulmonary Arterial Hypertension (PAH)

- Rare progressive disease affecting lung vasculature
  - 15-50 cases per million in US and Canada[1]
  - Incidence of 1-2 cases per million[1,2]
- Adults with PAH
  - Median survival of 2.8 years
  - Lung transplantation is the sole treatment option[3]



Pulmonary Vasculature Remodeling

Susceptibility:

Abnormal BMPR2 signaling[4]

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Vascular Remodeling:

Smooth muscle proliferation in large vessels and endothelial cell apoptosis in distal vessels[4,5]

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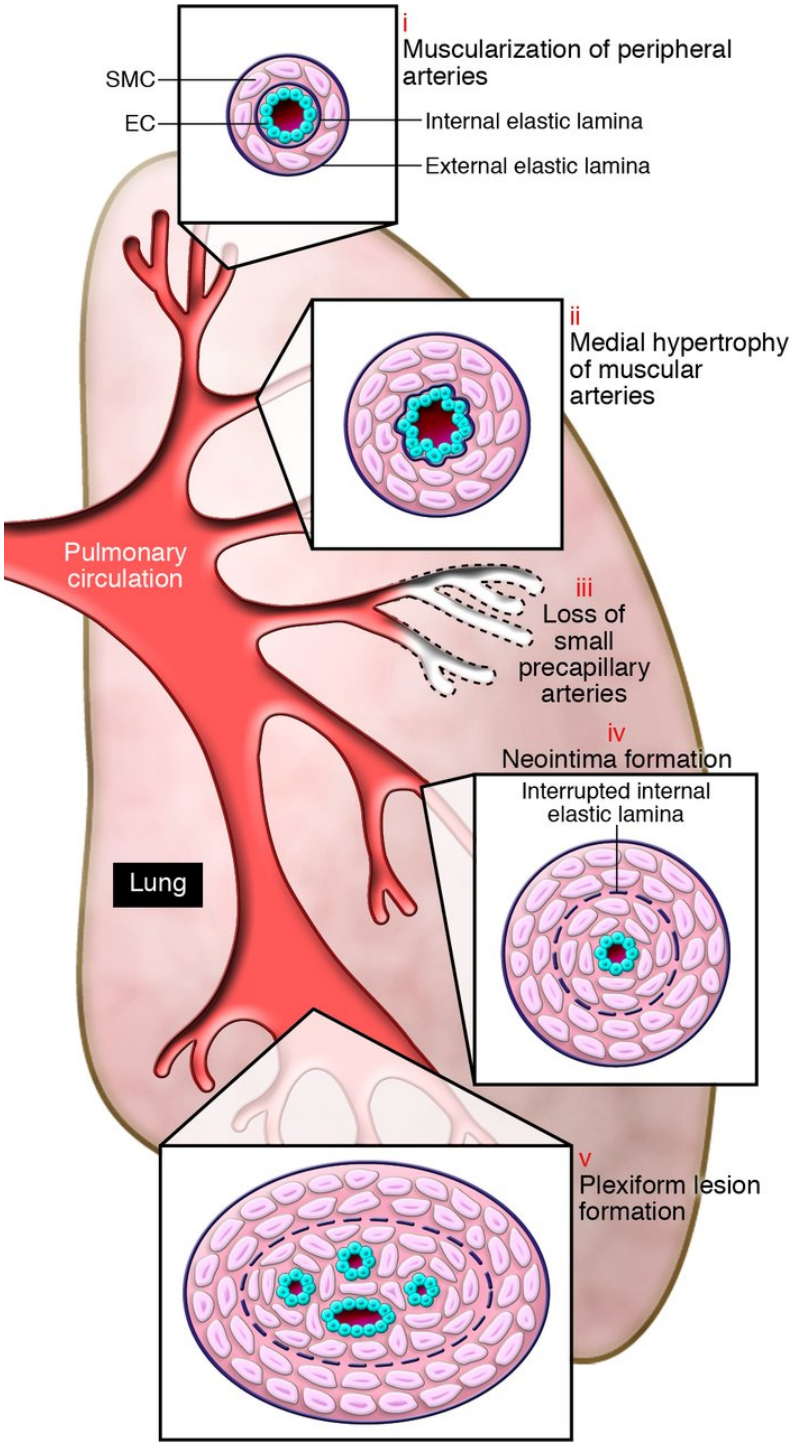
Disease Progression:

Endothelial cell proliferation in large vessels, neointimal growth and plexiform lesion[5]

(Rabinovitch, M – 2008)



Disease Implications

Hypertension in Pulmonary Artery

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Increased Resistance to Blood Flow

Pressure increases in the heart causing stress and damage to blood vessels and muscle[6]

⇩

Disease State

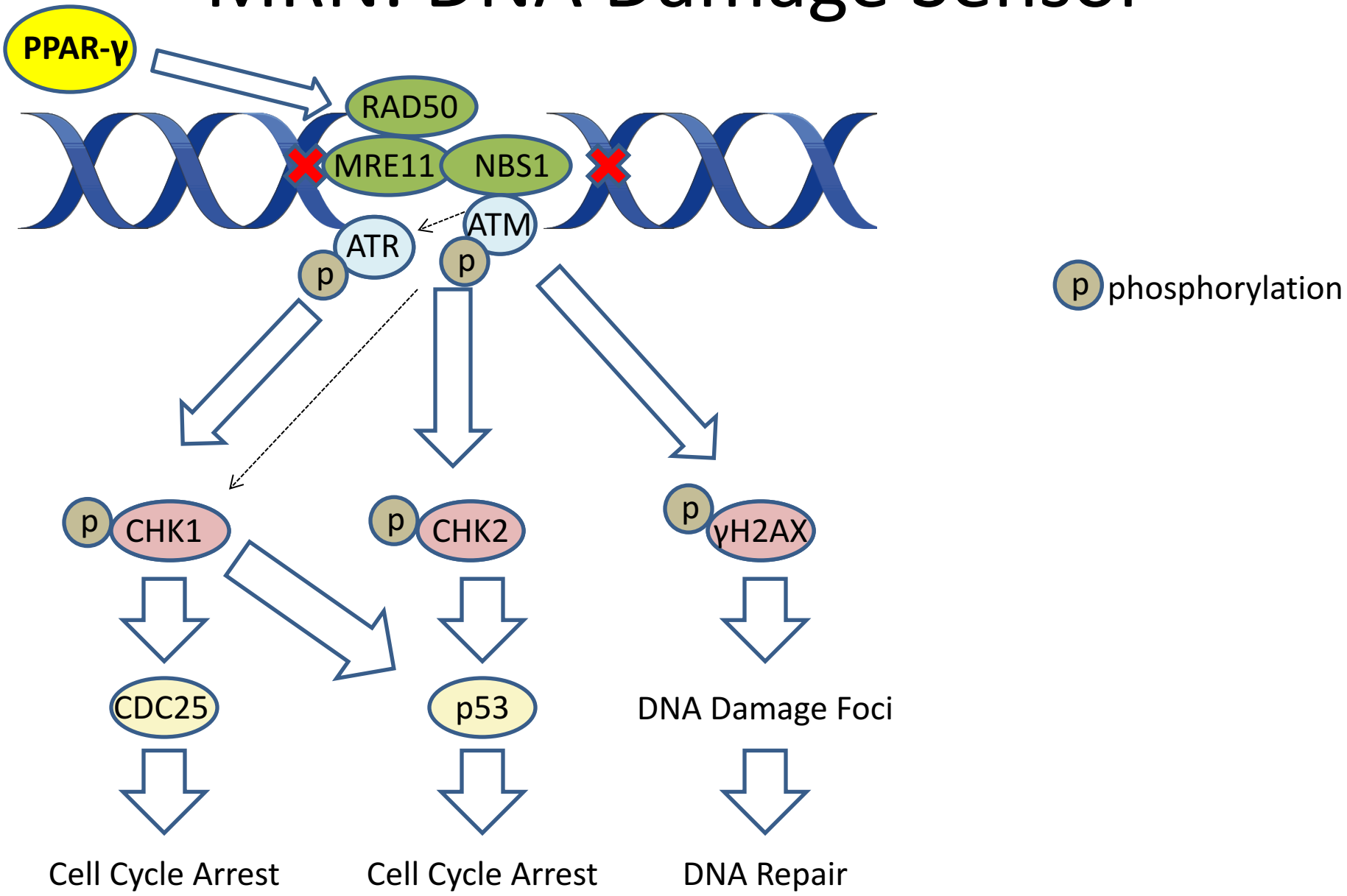
Right Heart Failure, COPD, CHF, Smoker's Lung, Diabetic Lung, Lung Cancer etc.[6]

(Rabinovitch, M – 2008)

Research Question

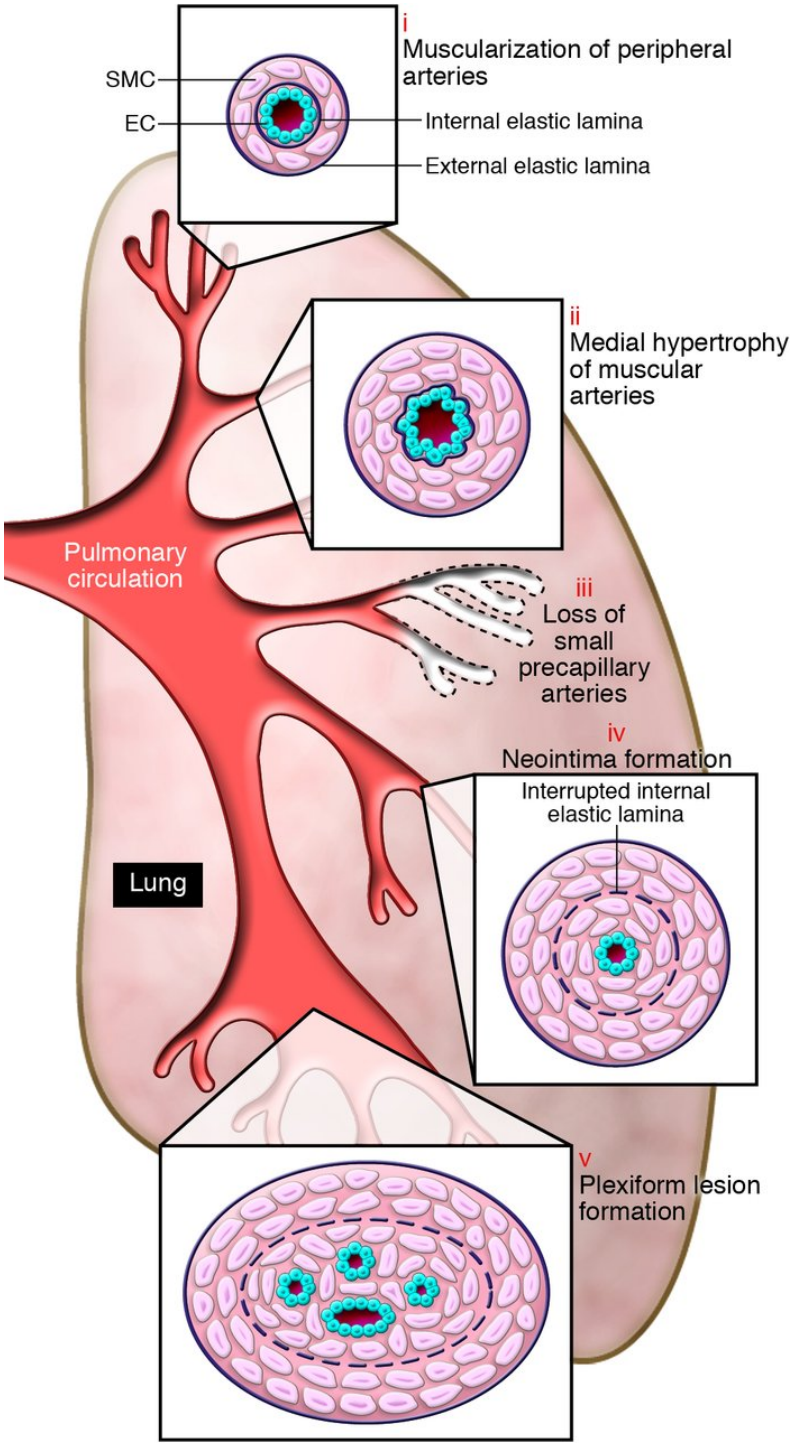
Can a reliable identification and/or therapeutic mechanism be derived for Pulmonary Arterial Hypertension?

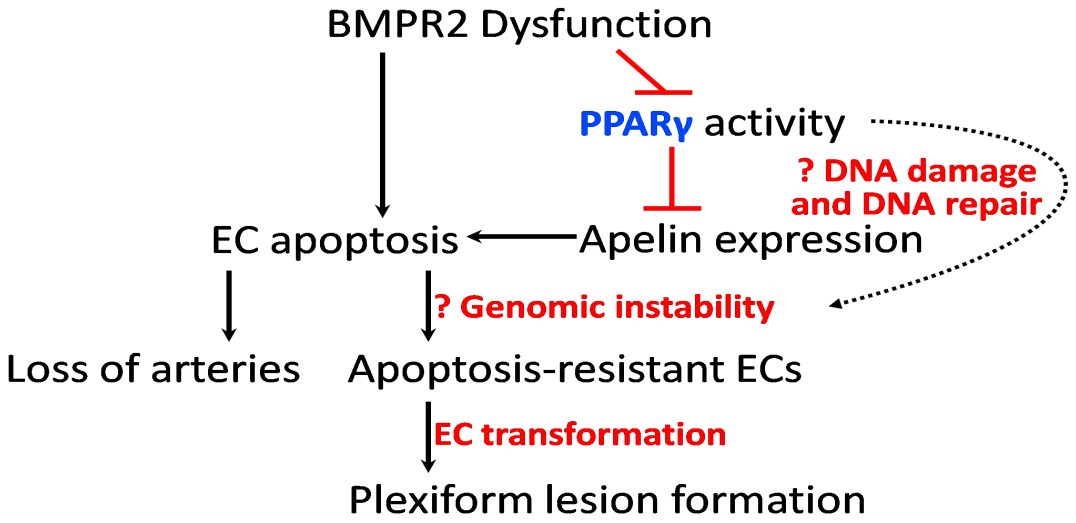
MRN: DNA Damage Sensor



(Ciccia, A – 2010)

Pulmonary Vasculature Remodeling



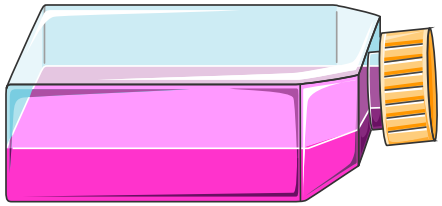


(Rabinovitch, M – 2008)

Hypothesis

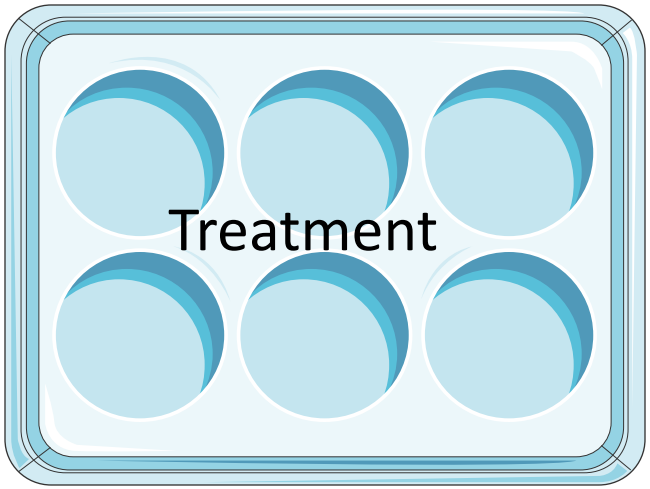
- The hypothesis states that PPAR-γ is required for normal DNA damage response in pulmonary arterial endothelial cells (PAEC).
- **Specific Aim 1:**
- To establish drug induced MRN mediated DNA damage response in 293T cells and PAEC.
- **Specific Aim 2:**
- To determine whether PPAR-γ inhibition prevents the activation of MRN mediated DNA damage response.

Methods



293T or
PAEC

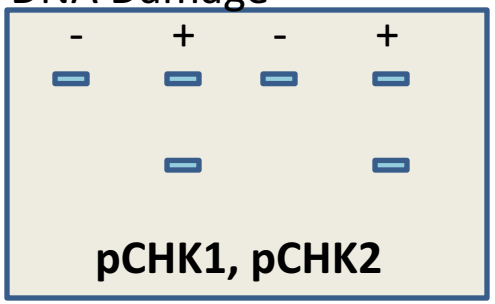
Seeding

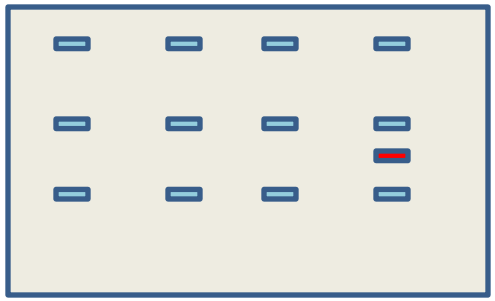


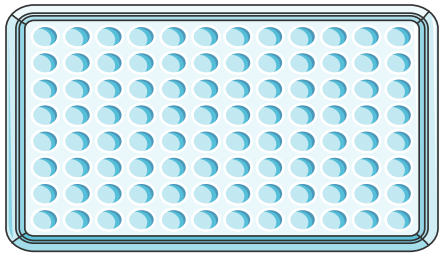
DNA Damaging Agents:
Doxorubicin
Hydroxyurea
Lipopolysaccharides

RIPA Buffer
Protein
Extraction

DNA Damage







Western Blot

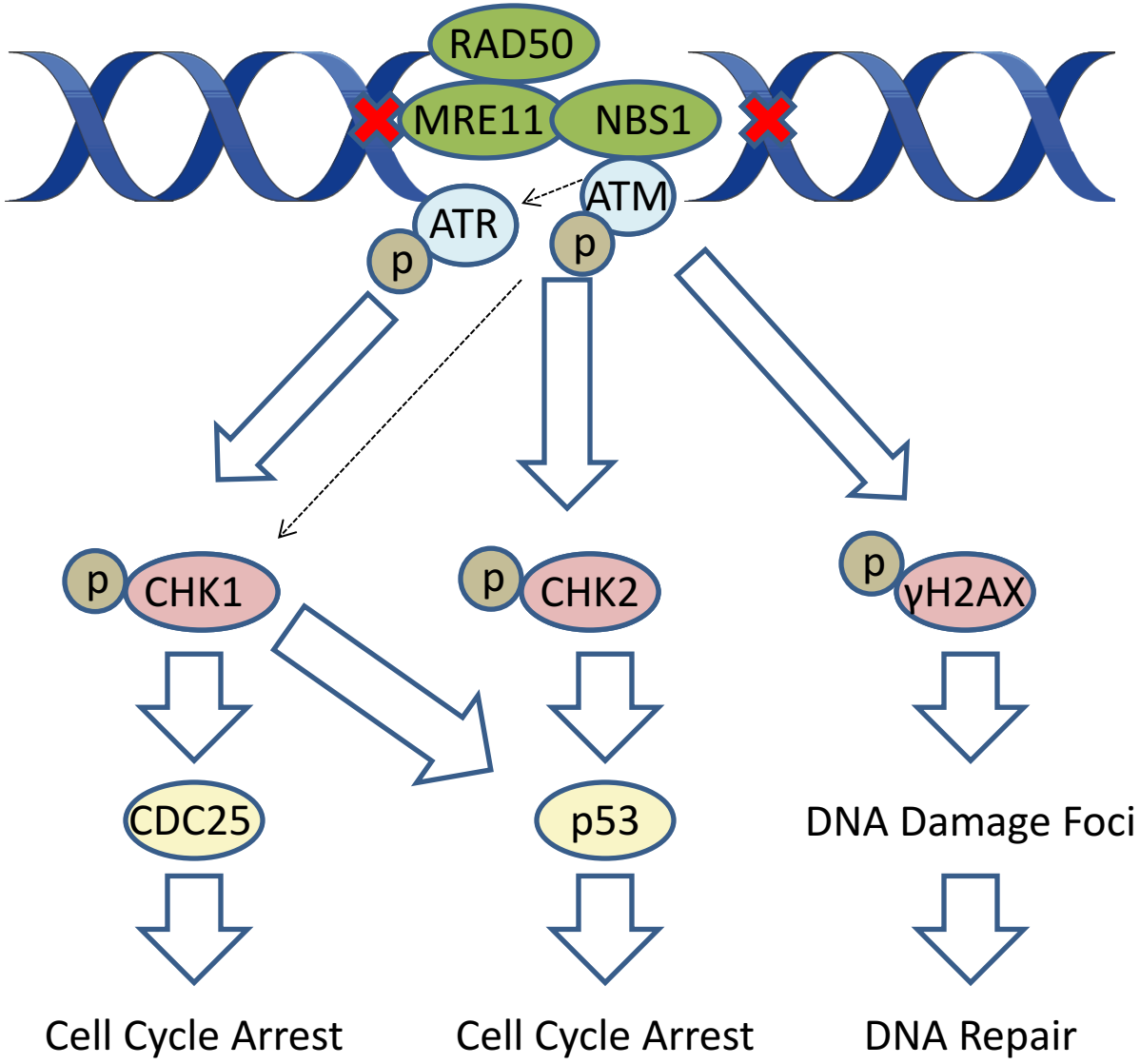
SDS PAGE

BCA Protein Assay

Drugs Used to Treat Cells

1. Doxorubicin (DoxR)
   - Alters DNA Structure[3]
2. Hydroxyurea (HU)
   - Deletion Mutation[3,4]
3. Lipopolysaccharide (LPS)
   - Free Radical Induction[5]

MRN: Linked to BMPR2 Repair



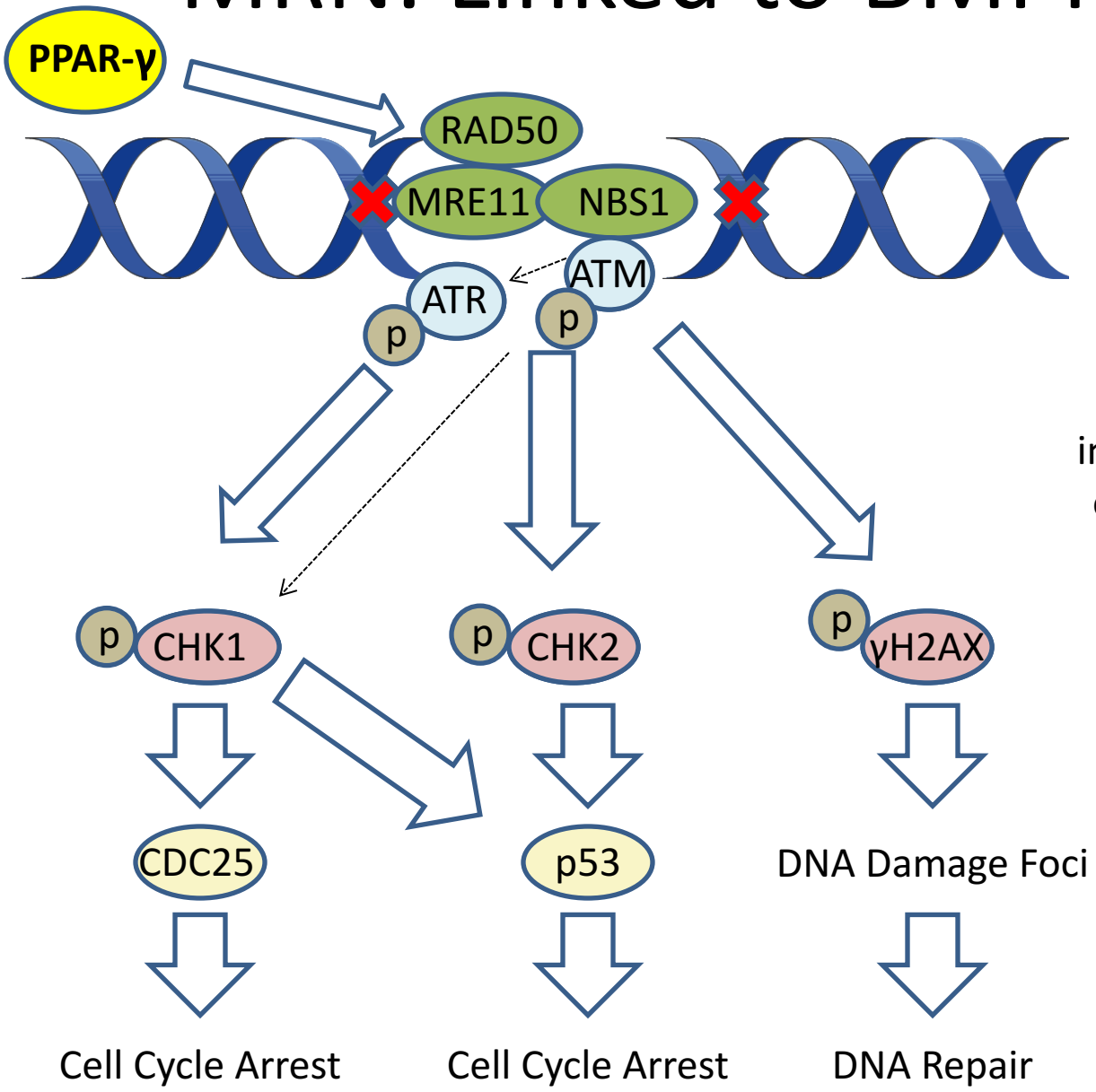
Specific Aim 1:
To establish drug induced MRN mediated DNA damage response in 293T cells and PAEC.

Increased pCHK1/pCHK2

Type of DNA Damage?

(Ciccia, A – 2010)

MRN: Linked to BMPR2 Repair

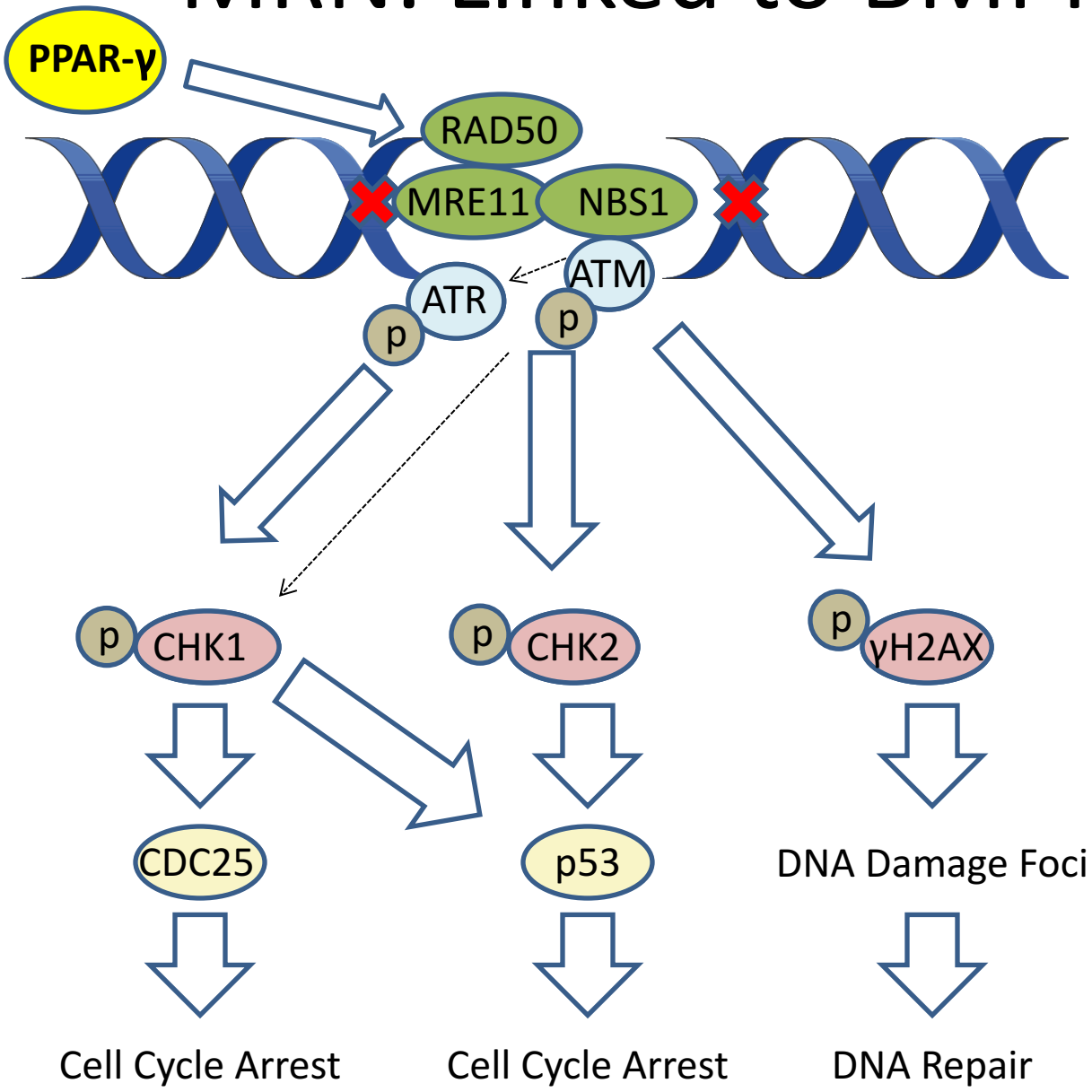


Specific Aim 2:
To determine whether PPAR-γ inhibition prevents the activation of MRN mediated DNA damage response.

Decreased pCHK1/pCHK2

(Ciccia, A – 2010)

MRN: Linked to BMPR2 Repair



Imaging:
DNA Damage Foci imaging and quantification

(Ciccia, A – 2010)

Timeline

- June – September
  - Specific Aim 1
    - Establish MRN mediated DNA damage response
- October – January
  - Specific Aim 2
    - Determine if PPAR-γ is linked to DNA damage response
- January – February
  - Image cell damage sites : time permitting

Conclusions

- PPAR-γ is a highly conserved molecule across various species lineages.
  - Well documented evidence of critical function in numerous organisms[8]
- Numerous agonists are well characterized
  - If PPAR-γ is linked to PAH, a therapeutic mechanism could be derived[9]

References

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