Gene-Environment Interactions In Hereditary Colorectal Cancer Risk

Fatimah Almousawi[1], Dr. Andrew Buermeyer[2]

BioResource Research Multidisciplinary Program[1]

Department of Environmental and Molecular Toxicology[2]

Oregon State University,

Corvallis, Oregon

Cancer Worldwide

Figure Source: Executive Healthcare Management

- More than ADIS, tuberculosis, and malaria combined
  - Union for International Cancer Control (UICC)

- By 2030, the annual number of cancer deaths may be as high as 13.2 million people
  - National Cancer Institute (NCI), 2012

Cancer Worldwide

Figure Source: Executive Healthcare Management

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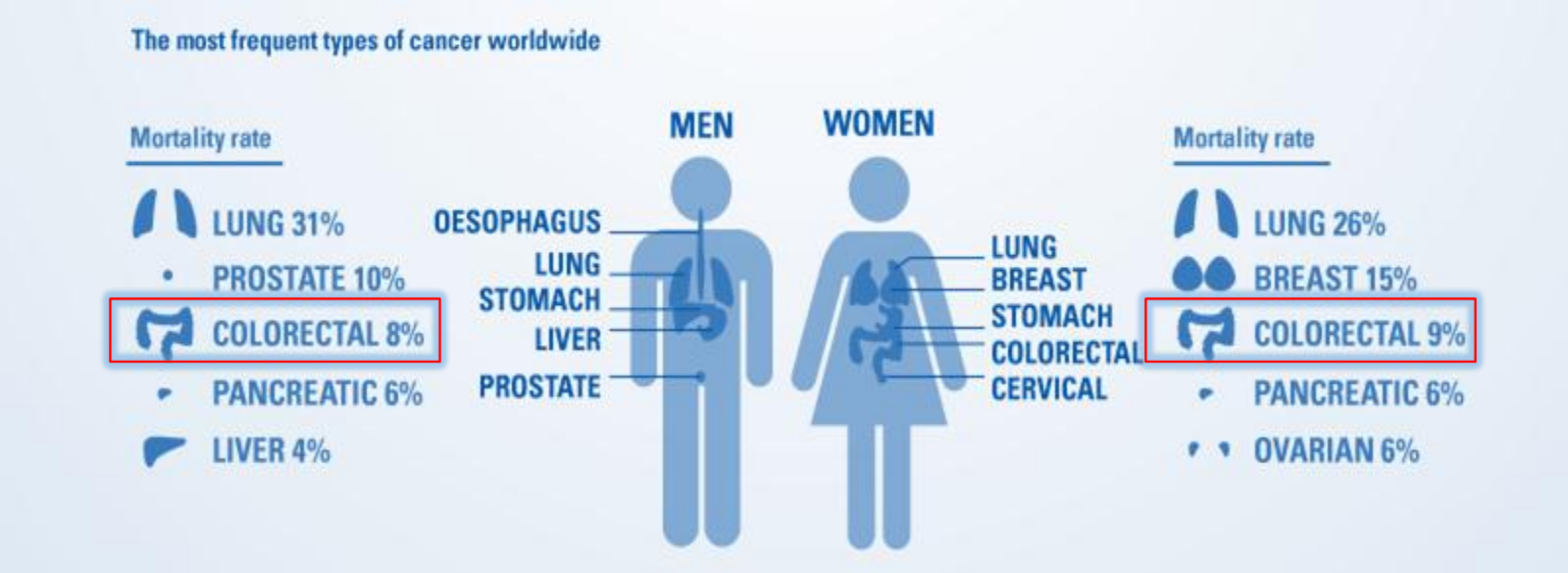
Colorectal Cancer (CRC)



- 143, 460 new colorectal cancer cases and about 51, 690 deaths in the U.S. in 2012. (ACS, 2012)
- Approximately $12.2 billion is spent in the United States each year on colorectal cancer treatment (NCI, 2012)

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Figure Source: Executive Healthcare Management

CRC Early Detection is Vital

- Five-year survival rates may be as high as 74%, or as low as 6% (ACS, 2012)

- Effective prevention depends on full understanding of individual risk factors

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Factors that increase cancer risk

- Mutations in critical genes in DNA repair pathways
  - Mismatch repair (Lynch Syndrome)
  - Nucleotide excision repair (XP Syndrome)
- Exposure to carcinogens
  - Polycyclic aromatic hydrocarbon (PAH)

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Polycyclic aromatic hydrocarbon (PAHs)

- Ubiquitous carcinogens
- Result from the incomplete combustion of organic compounds
- Exposure: fossil fuel processing, smoking, grilling food

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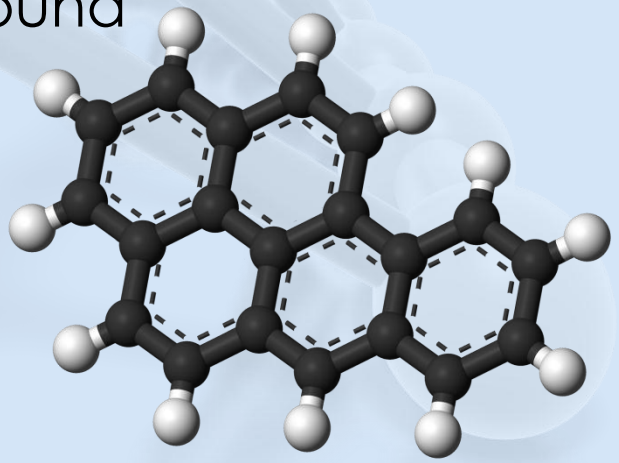
Benzo[a]pyrene (B[a]P)

- Classified by EPA as group 1 carcinogen
- Detected in air, water, food and soil
- World Health Organization (WHO): amounts of B[a]P should not exceed 10 ppb in foods
- Some barbecued meats have been found to contain up to 30 ppb of B[A]P.



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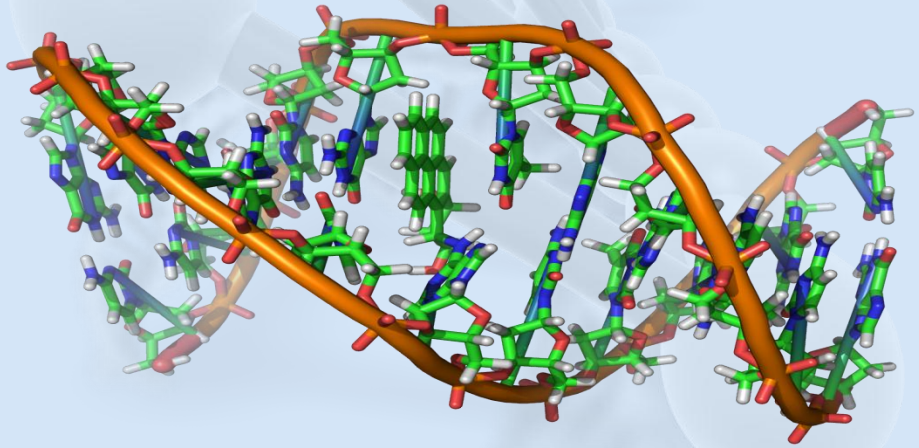
PAH metabolites

- PAHs are metabolically activated intracellularly to reactive diol epoxides (DE)
- DE covalently binds to the DNA and forms stable adducts
- These adducts initiate mutation and eventually lead to cancer.



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Mismatch Repair

- Provides several highly conserved genetic stabilization functions
- An excision/resynthesis process with 4 phases:
- MLH1 & PMS2 (MutLa) or MSH2 and MSH6 (MutSa)

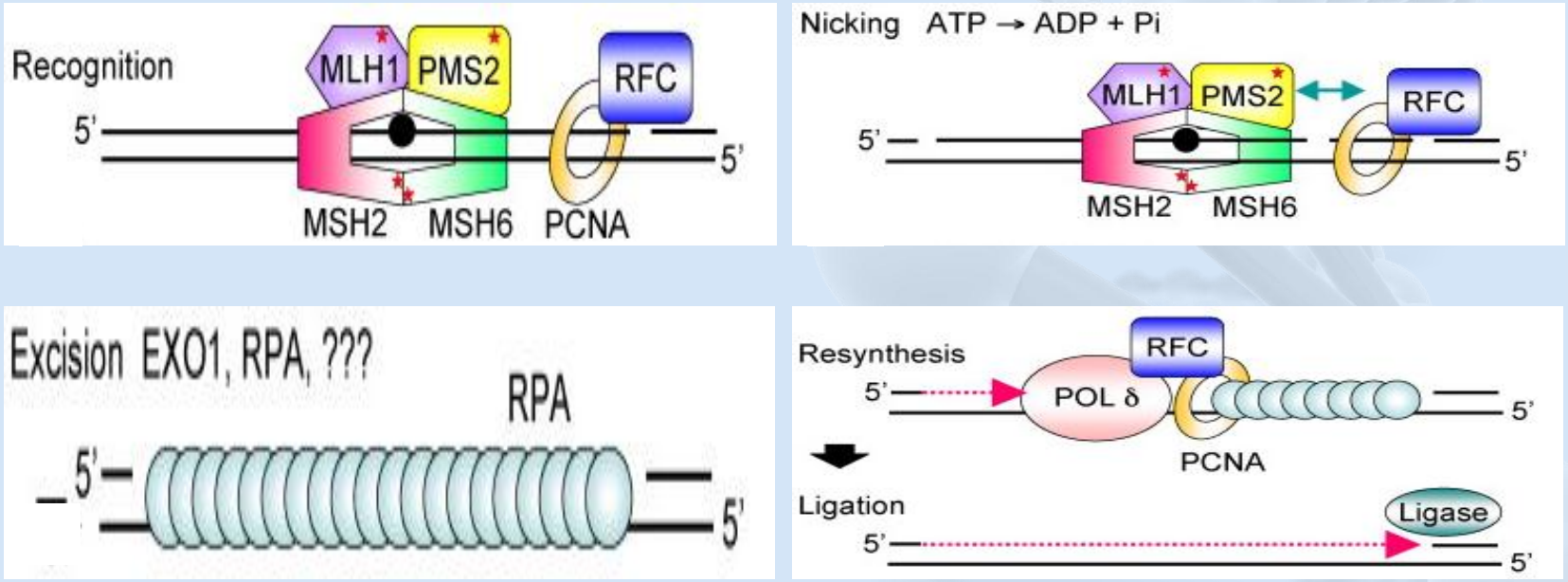

Figure source: (Laat, 1999)

Mutator Phenotype

Mutations are a driving force behind cancer development

Mutated MMR genes

Replication errors bypass defective MMR systems

Mutations inactivate tumor suppressor genes

Heterozygous and homozygous MLH1 mutant mice had reduced longevity compared to their wild-type littermates

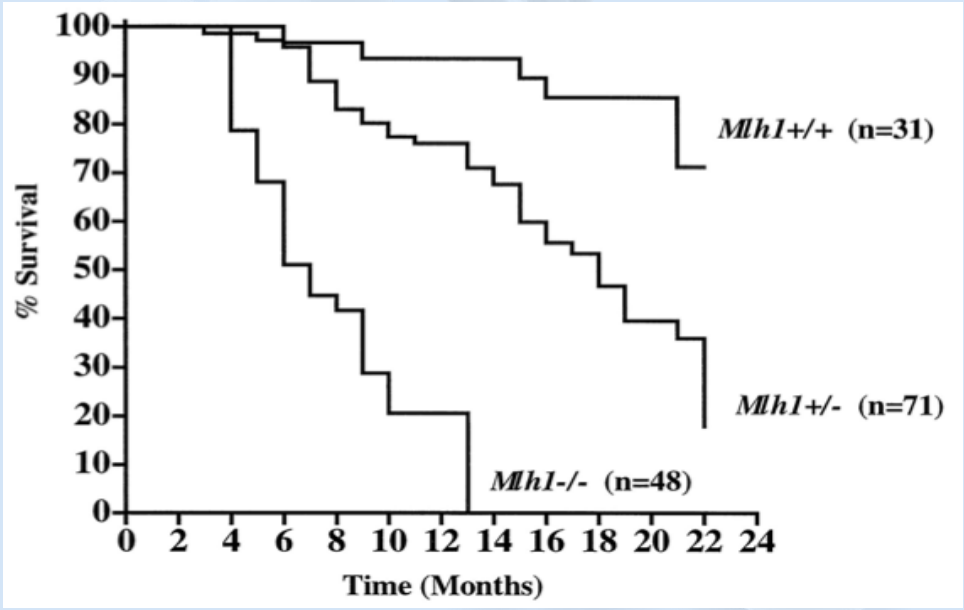

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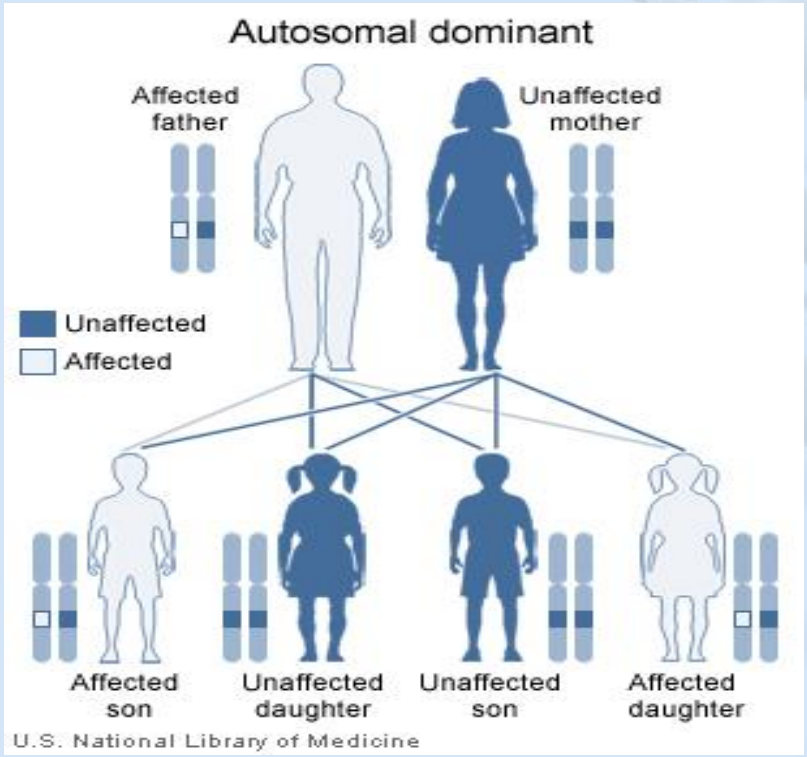
Figure source: Edelmann, 1999

Lynch Syndrome

- The most common form of hereditary colon cancer
- Autosomal dominant genetic condition
- Mutation in one or more of MMR genes:
  MLH1 & PMS2 (MutLa) or MSH2 and MSH6 (MutSa)



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Nucleotide Excision Repair (NER)

- Highly versatile and sophisticated DNA damage removal pathway
- Recognize and repair DNA damages such as damage induced by PAH exposure
- "Cut and paste" process
- Excise and remove short fragments of DNA that are 24 to 32 nucleotides with the damaged base

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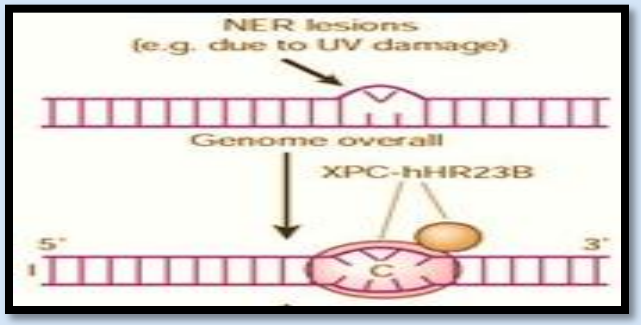
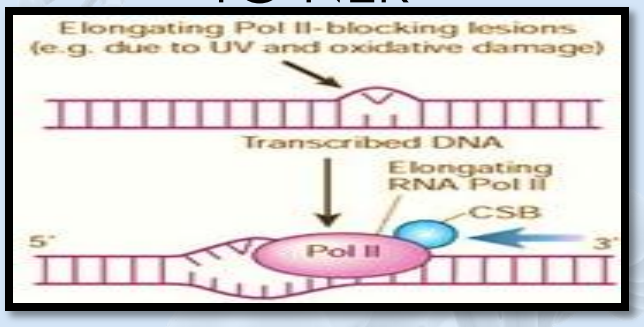
Nucleotide Excision Repair (NER)

- Two sub-pathways: global genome NER (GG-NER)
  transcription-coupled NER (TC-NER)

GG-NER



TC-NER



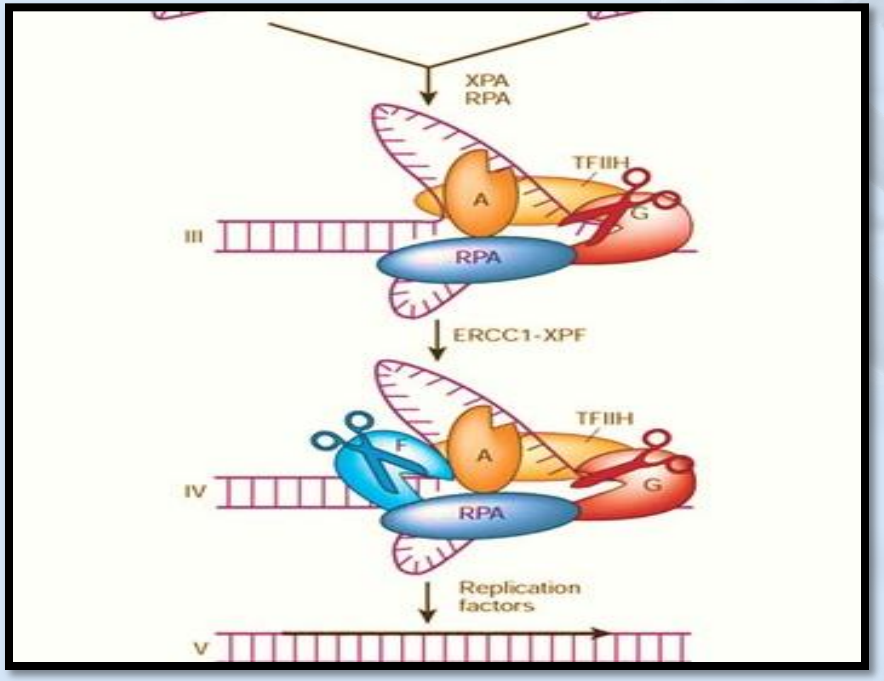


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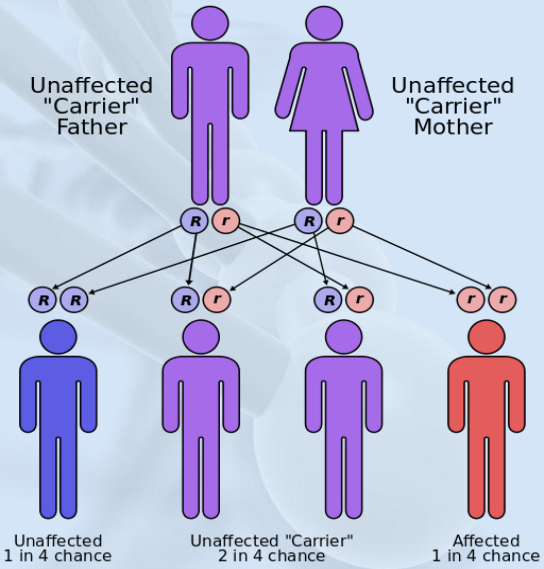
Figure soruce: Hoeijmakers, 2011

NER Deficiencies

- Deficiencies in the NER pathway cause human autosomal recessive diseases

- Xeroderma pigmentosum (XP)

  - extreme sensitivity to sunlight and great increase UV-induced mutation rates



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NER Deficiencies

- NER deficient cells are very sensitive to killing by exposure to PAH

- Transgenic mice lacking NER exhibit dramatically elevated cancer rate

  - NER is an important pathway for protection against cancer associated with exposure to PAH

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Study Overview

- Exposure to certain environmental agents, such as PAHs, can increase risk of developing cancer

- DNA repair pathways are important for protection against the toxic effects of DNA damaging agents

  - deficiencies in important genes in DNA repair pathways are associated with increased risk of cancer

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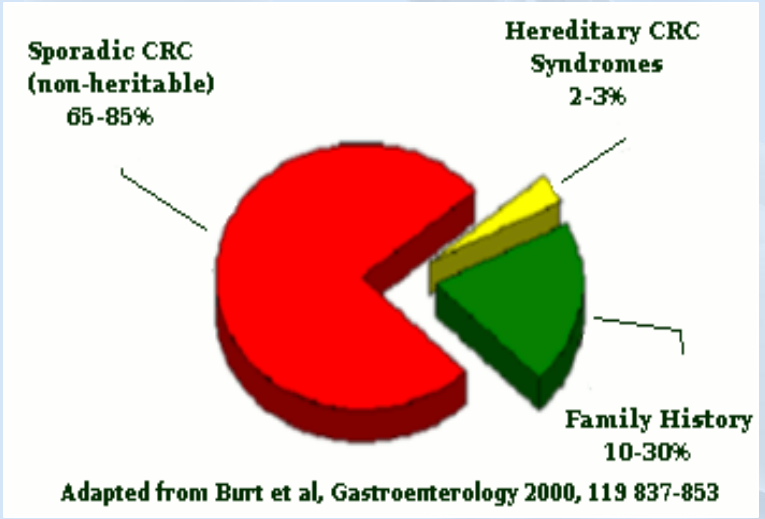
- Although Lynch syndrome is associated with increase risk of colorectal cancer
  - only for 3- 5% of all cases of colorectal cancer
- XP cases are more rare
- The genetic factors underlying the susceptibility in the general population are unknown



Hypothesis:

Partial deficiencies in multiple DNA repair pathways (MMR and NER), would interact synergistically to increase the risk of genotoxic effects of carcinogenic exposure in the general population.

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To test the hypothesis:

Create an experimental system to model the individuals who have multiple, partially deficient DNA repair pathways

Creating isogenic cell cultures with reduced gene expression for either MMR (MLH1 and MSH2) or NER (XPA)

Goals:

- Generate cells with deficiencies in both MMR and NER
- Measure the effect of the combined genetic deficiencies on PAH-induced genotoxicity

Prediction:

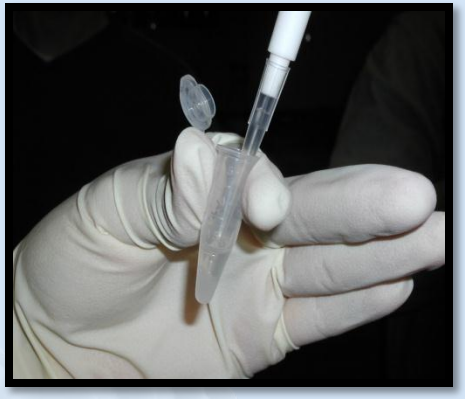
Cells with combined deficiencies MMR and NER would exhibit a synergistic increase protein accumulation in culture cells

Methods

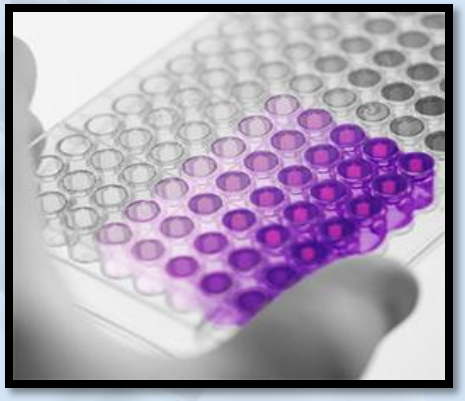
Experimental Procedure:

HeLa cells

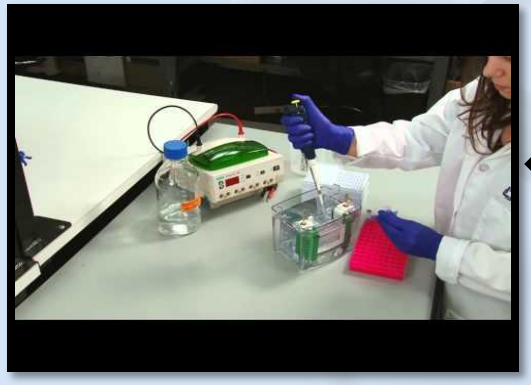
siRNA transfection



Whole Cell Protein Lysates



BCA assay



Immunoblot

PAH exposure

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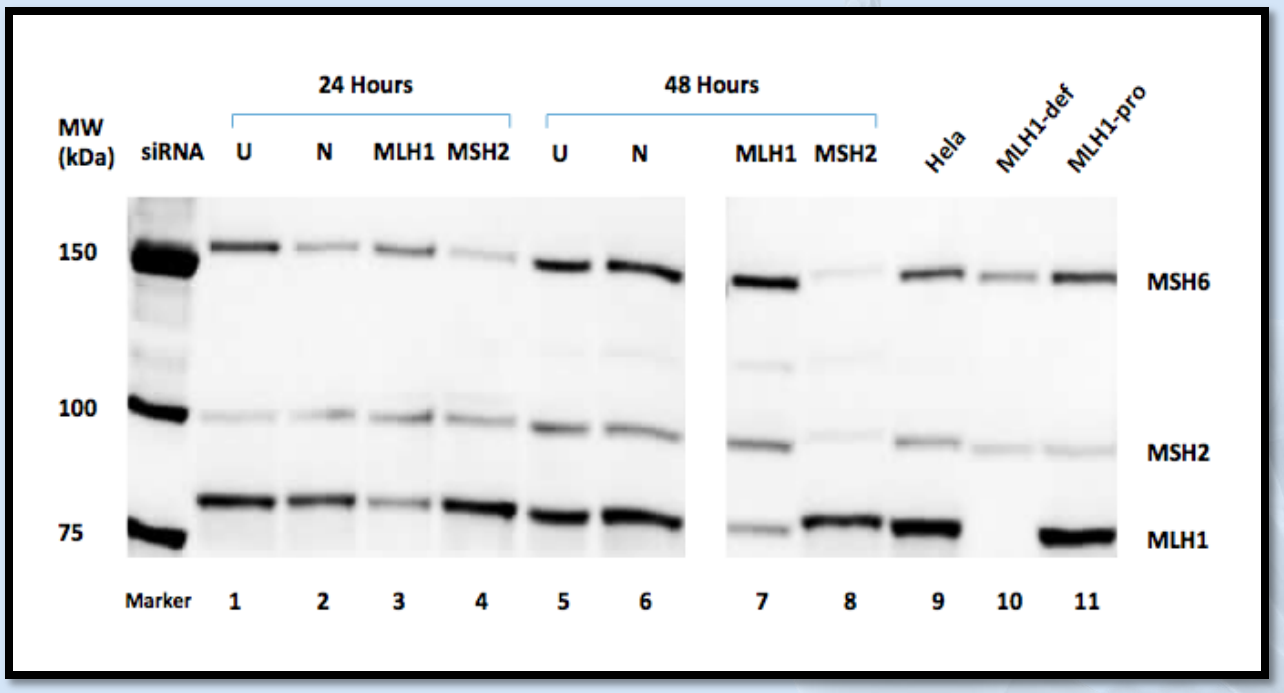
Results and Discussion

Testing the extent of inhibition of MLH1 and MSH2 protein accumulation



The Molecular weight:

- MSH2: 104.7 kDa
- MSH6: 160 kDa
- MLH1: 84.6 kDa

- Unexplained variability in band intensities in the 24hr U and N samples versus the 48hr samples
- Successful reduction in MLH1accumlation
- Inconsistent reduction for MSH2 accumulation

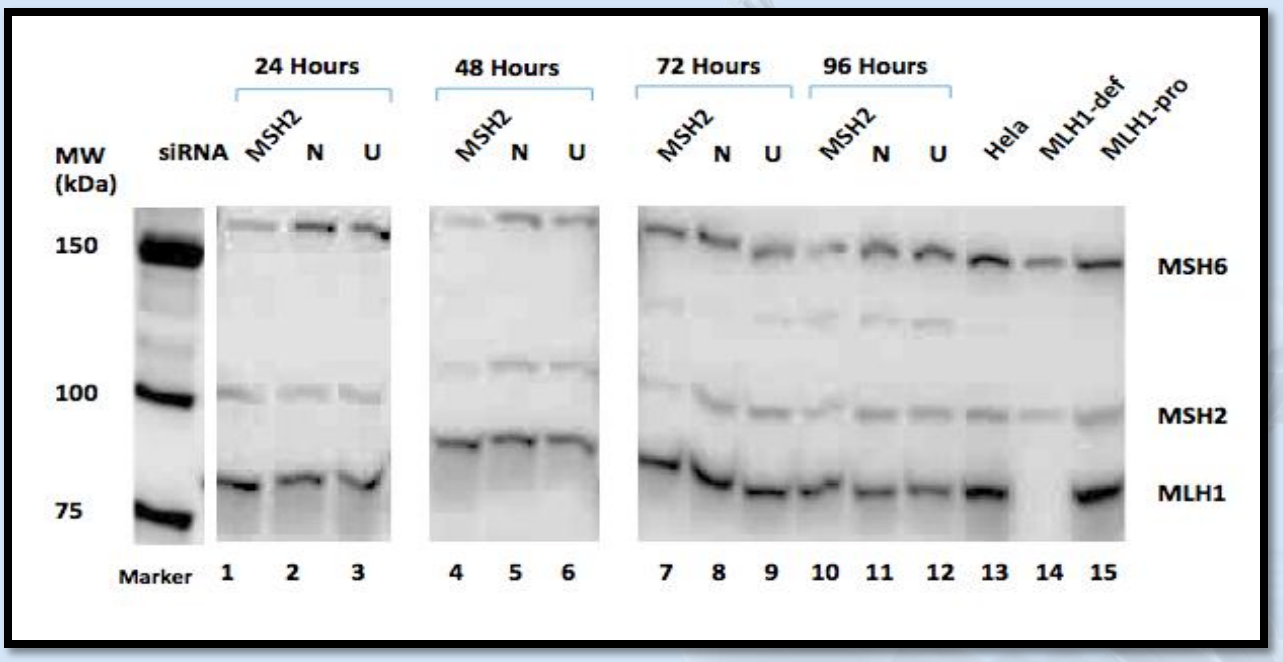
In conclusion:

Initial results suggested that the siRNA successfully reduced the accumulation of MLH1 and MAH2 proteins, in particular at 48-hour post-transfection

Questions:

- To what extent the proteins were reduced?
- The maximum degree of inhibition possible with our siRNA?
- What happen at different time points and conditions?

Analyzing the time course for MSH2 inhibition



- MLH1 bands were consistent
- No MSH2 reduction at 24hr compared by the controls
- MSH2 bands' intensities were reduced at 48hr
- MSH2 Reduction is difficult to confirm at 96hr

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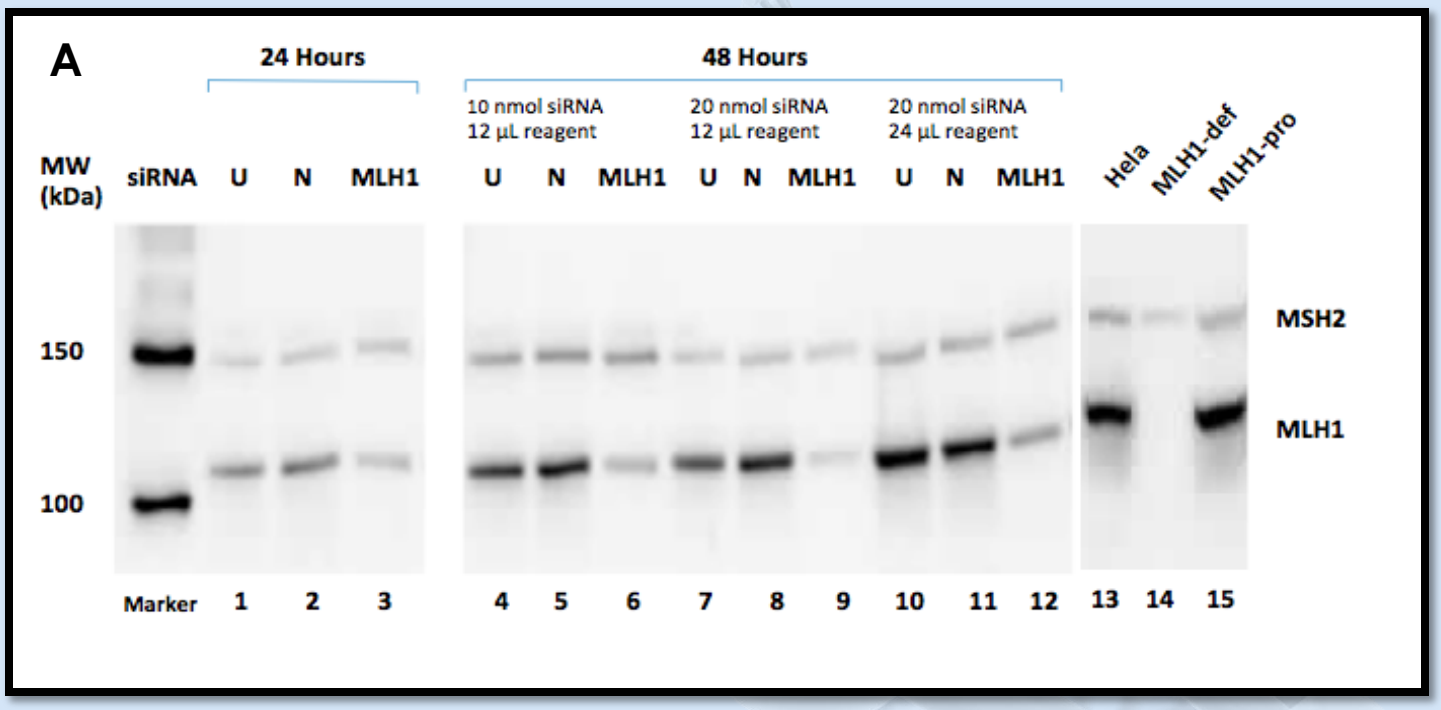
Conclusion:

- The inhibition of MSH2 was most effective at 48 and 72hr post-transfection

- These two time points would be most useful for analysis of cells with engineered MMR-deficiencies in future experiments

Analyzing the time course for MLH1 inhibition at several different conditions (A)



- The inhibition was more significant at 48hr then 24hr
- Doubling siRNA amounts had no apparent different compared to the initial amount of siRNA used

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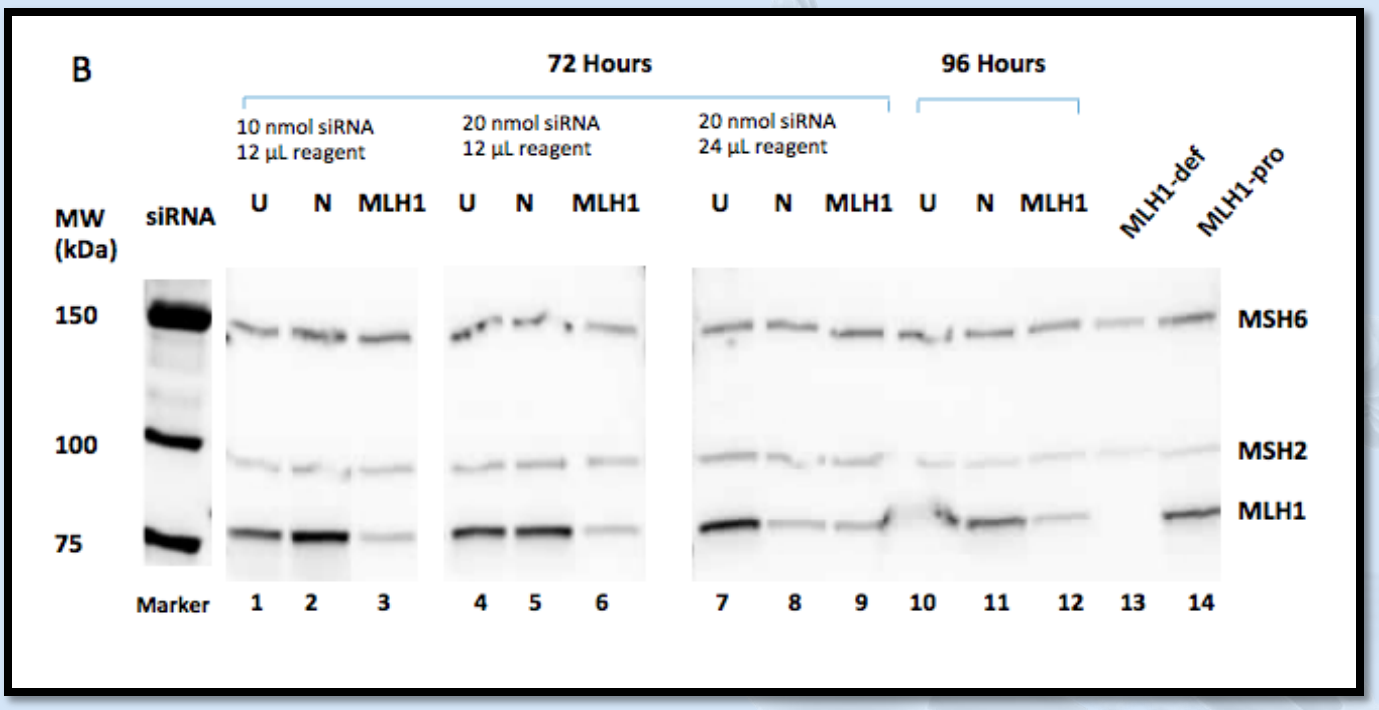
Analyzing the time course for MLH1 inhibition at several different conditions (B)



- The increased amount of siRNA with either the same or doubled transfection reagent did not show greater MLH1 inhibition
- MLH1 band intinsities was reduced, but not as significantly as the reduction observed at 48hr and 73hr

Conclusion:

- The increased siRNA was not more effective in inhibiting the accumulation of MLH1
- A repeated experiment might be needed to confirm whether or not doubling the siRNA amounts lead to greater reduction of MLH1 accumulation
- Best time course for MLH1 inhibition is between 48 and 72hr post-transfection

Summary

- We were able to demonstrate a partial reduction in accumulation of MLH1 and MSH2 in cultured HeLa cells over multiple time points using the identified siRNA

- The project did not progress to testing the XPA-targeting siRNA in our cells

- Therefore, we are unable to test the effect of the combined deficiencies.

Future Investigations

- Test the efficiency of MMR pathway of the created cells to determine whether or not the degree of MLH1and MSH2 inhibition has affected the pathway

- Create cell lines with multiple XPA deficiencies

- Ultimately, expose all of the cell lines created to PAHs to measure the effect of combined deficiencies of PAH-induced mutagenesis

Benefits of Research

Our results confirm the potential utility of specific siRNA targeting either MSH2 or MLH1, and helped establish transfection conditions for their use in cultured HeLa cells

Acknowledgment:

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