

The Role of p53 and MDM2 Expression in Pediatric Rhabdomyosarcoma



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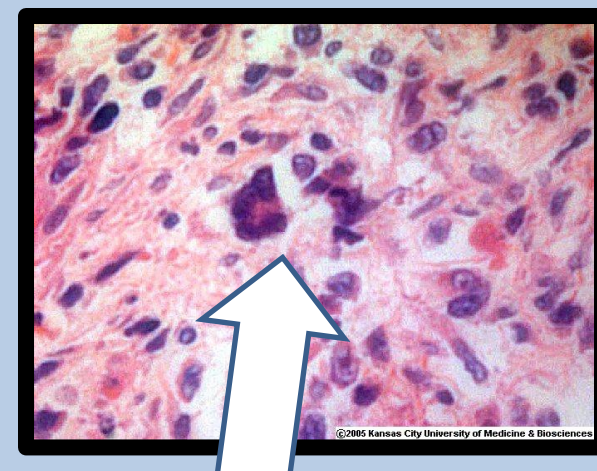
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1. Rhabdomyosarcoma (RMS) accounts for over half of the soft tissue sarcomas diagnosed in children.

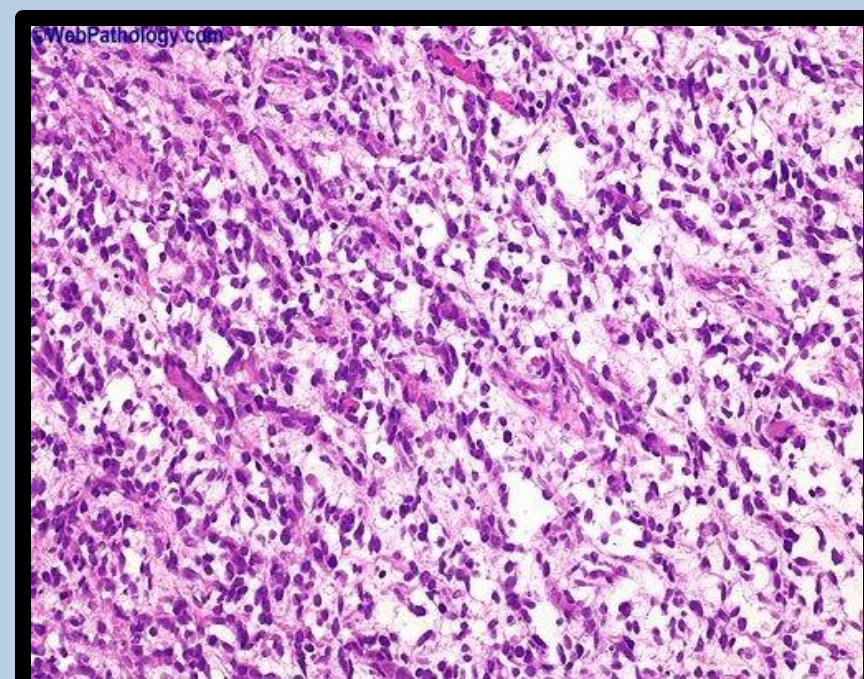


- 350 new cases diagnosed each year in US
- 70% of all cases are diagnosed in first 10 years of life
- Highly malignant and fast growing

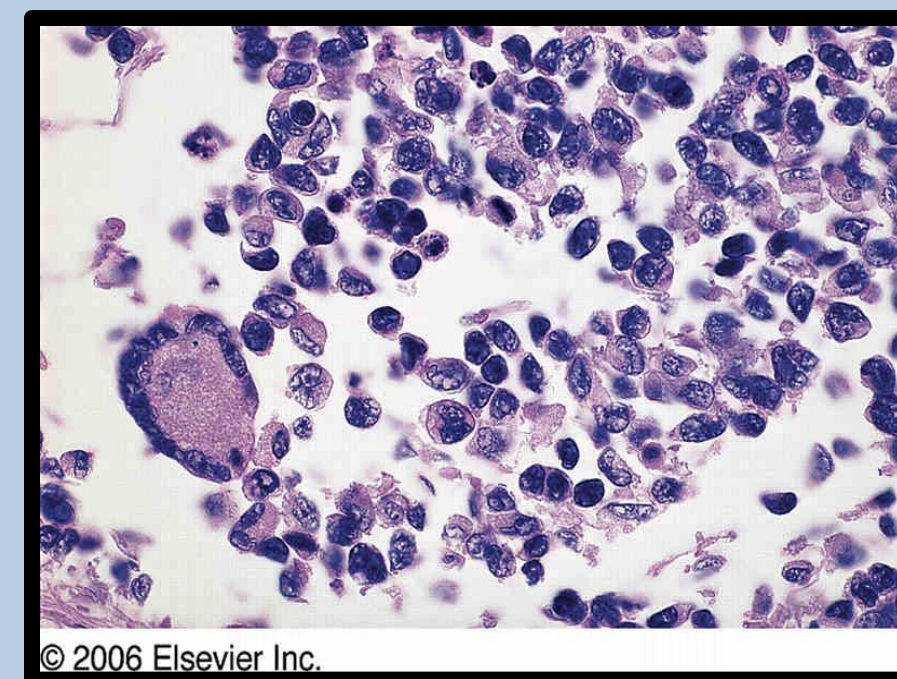


rhabdomyoblast

- Tumors found in head, neck, trunk, extremities, and genitourinary tract
- Hypothesis: The protein pathways associated with proliferation of tumor cells will be activated in p53-positive (mutant) tumors in both Alveolar RMS and Embryonal RMS.
- In the absence of p53 mutations, MDM2 (murine double minute 2) over-expression will inhibit wild type p53.



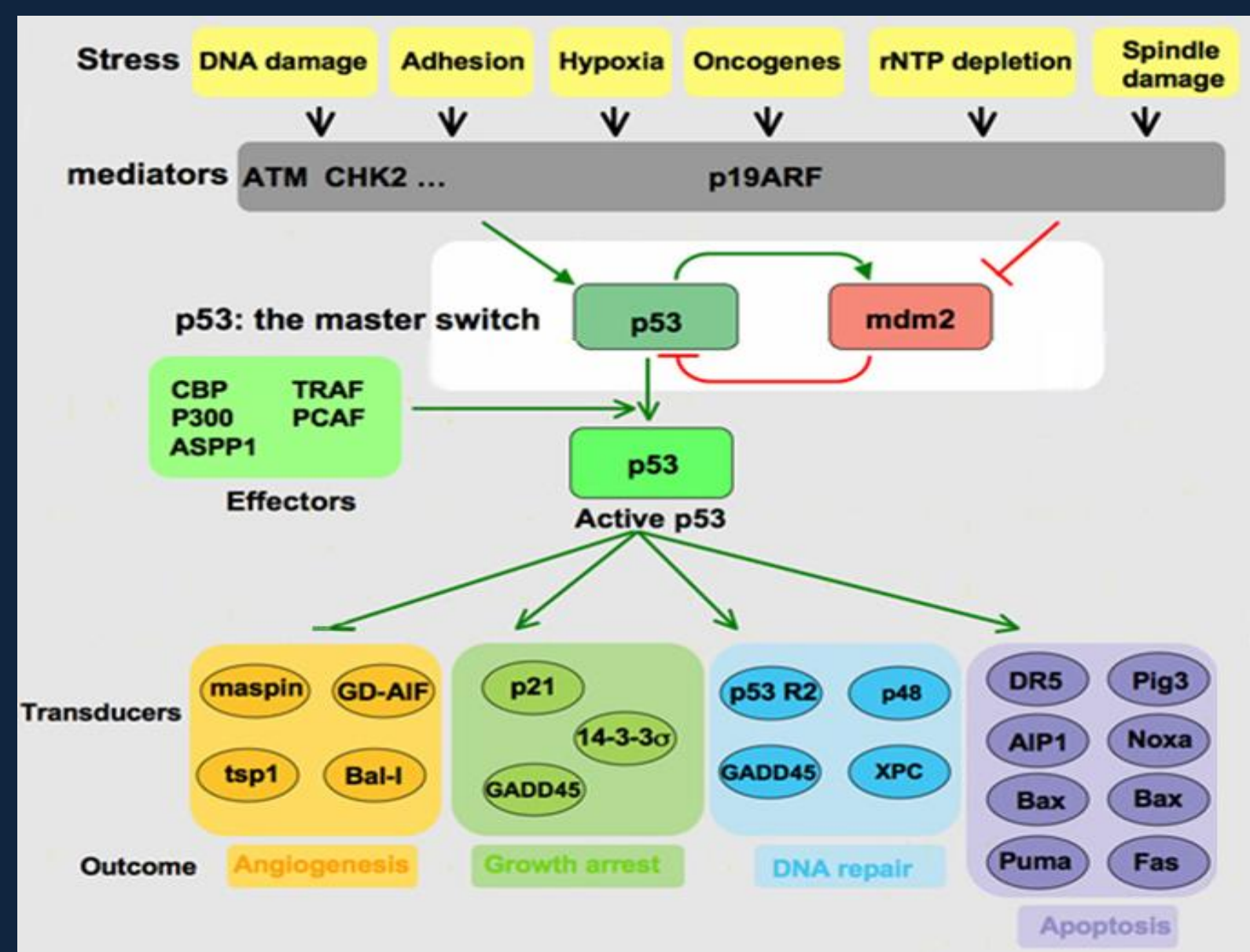
Embryonal RMS (ERMS)



Alveolar RMS (ARMS)

2. Exploring p53 and MDM2 protein expression in RMS tumors

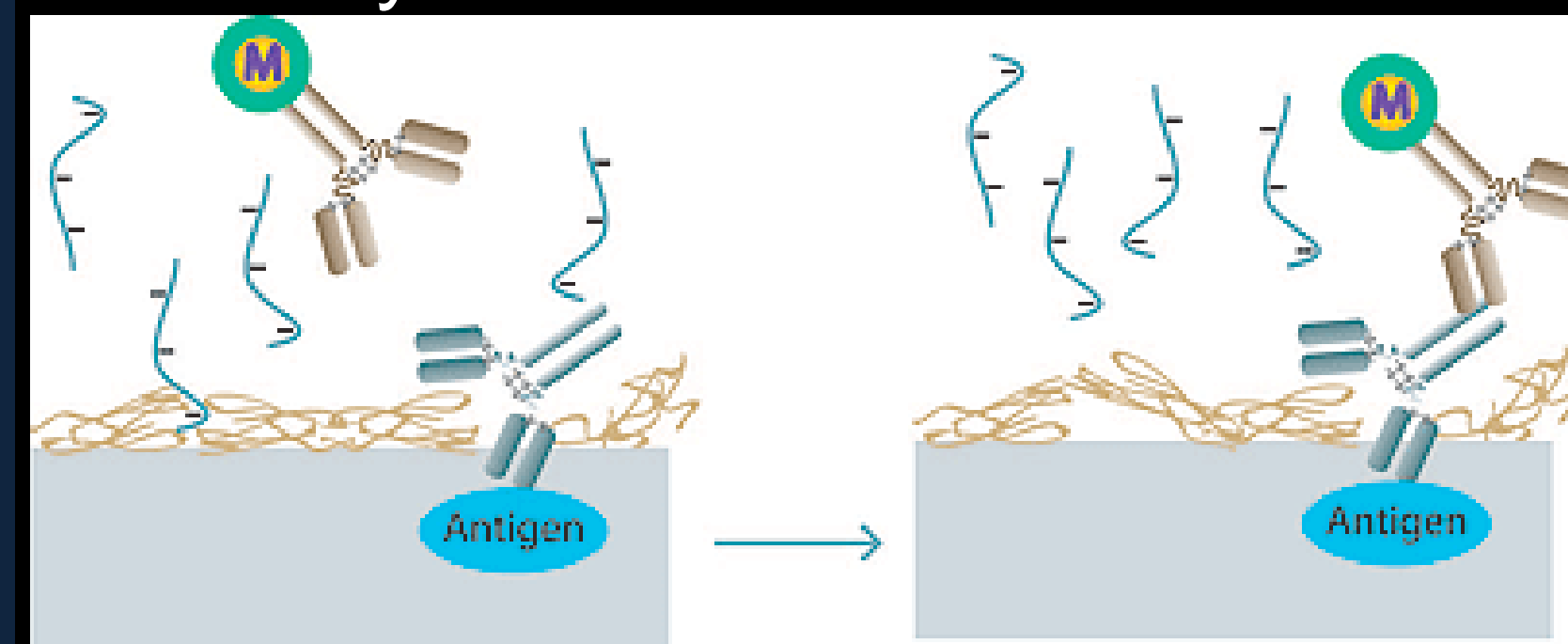
P53 Tumor Suppression Pathway



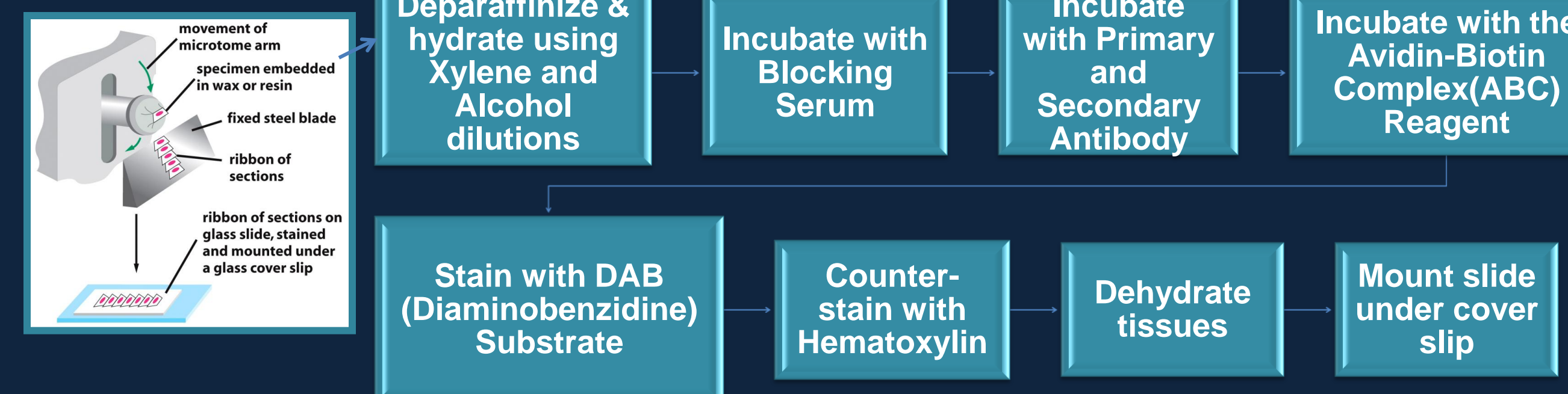
- Normal p53 directs damaged cells to DNA repair or apoptosis (programmed cell death)
- Increased MDM2 expression inhibits p53 function

3. What is immunohistochemistry and how does it work?

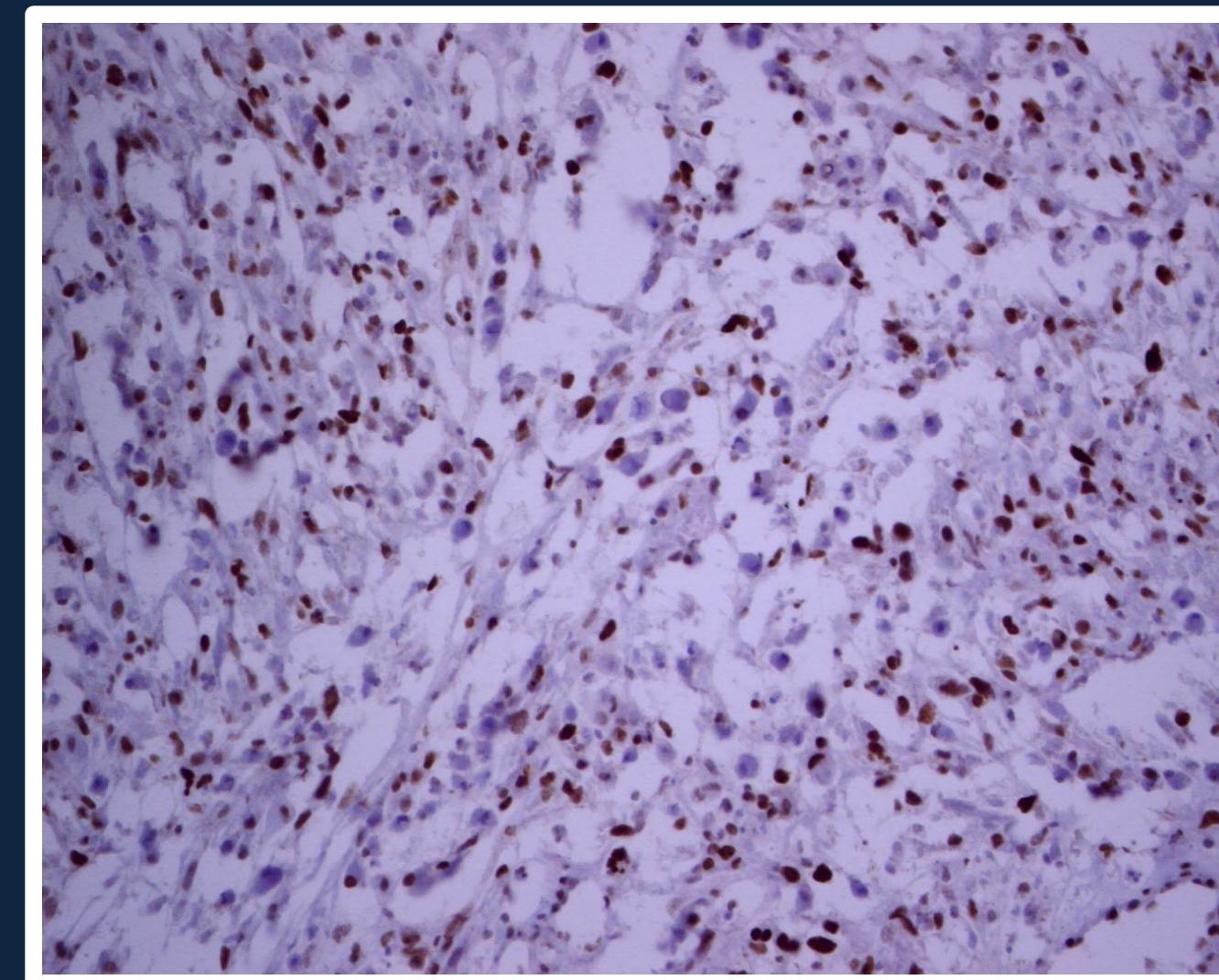
Antigen-Antibody Reaction



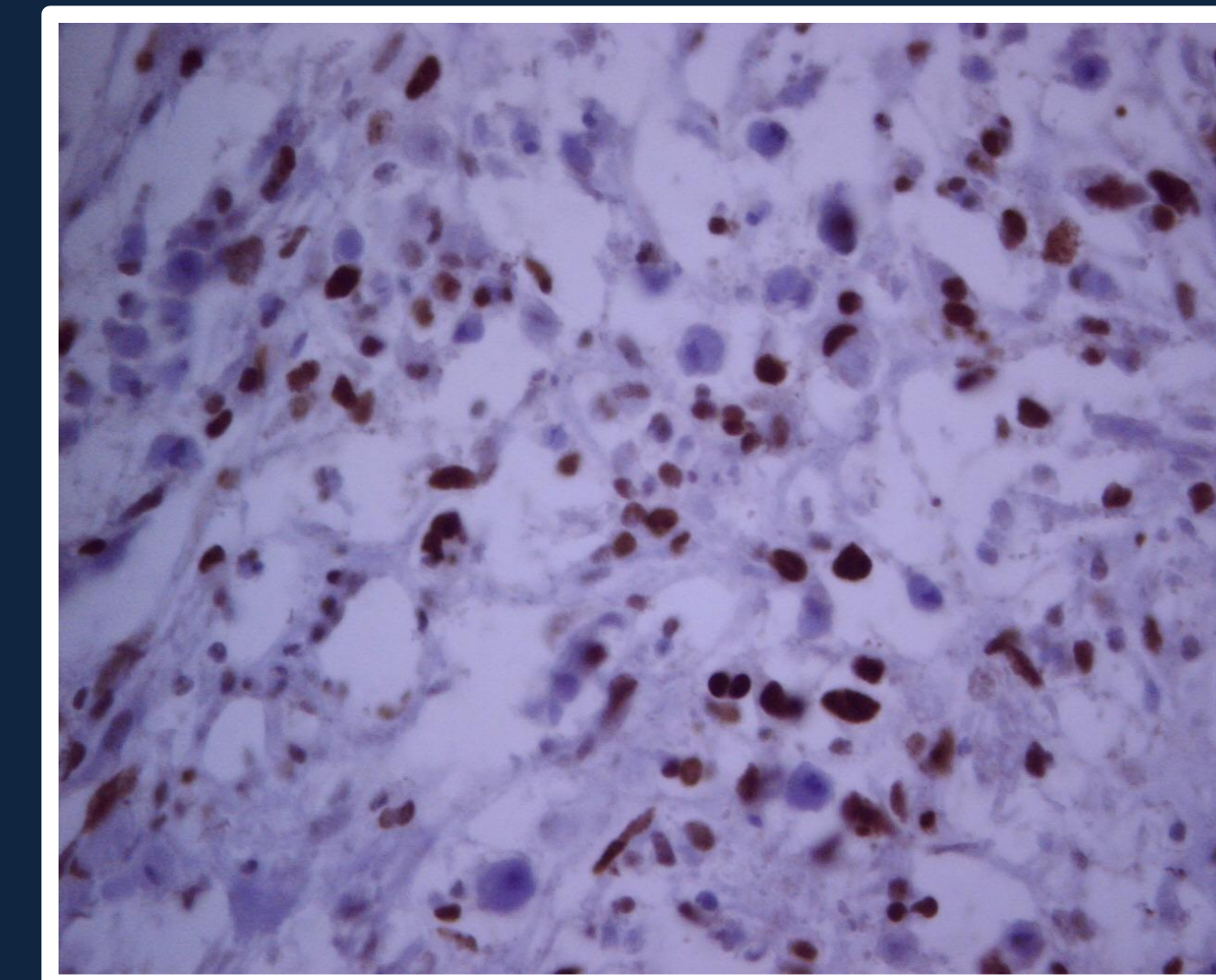
Immunohistochemistry Procedure



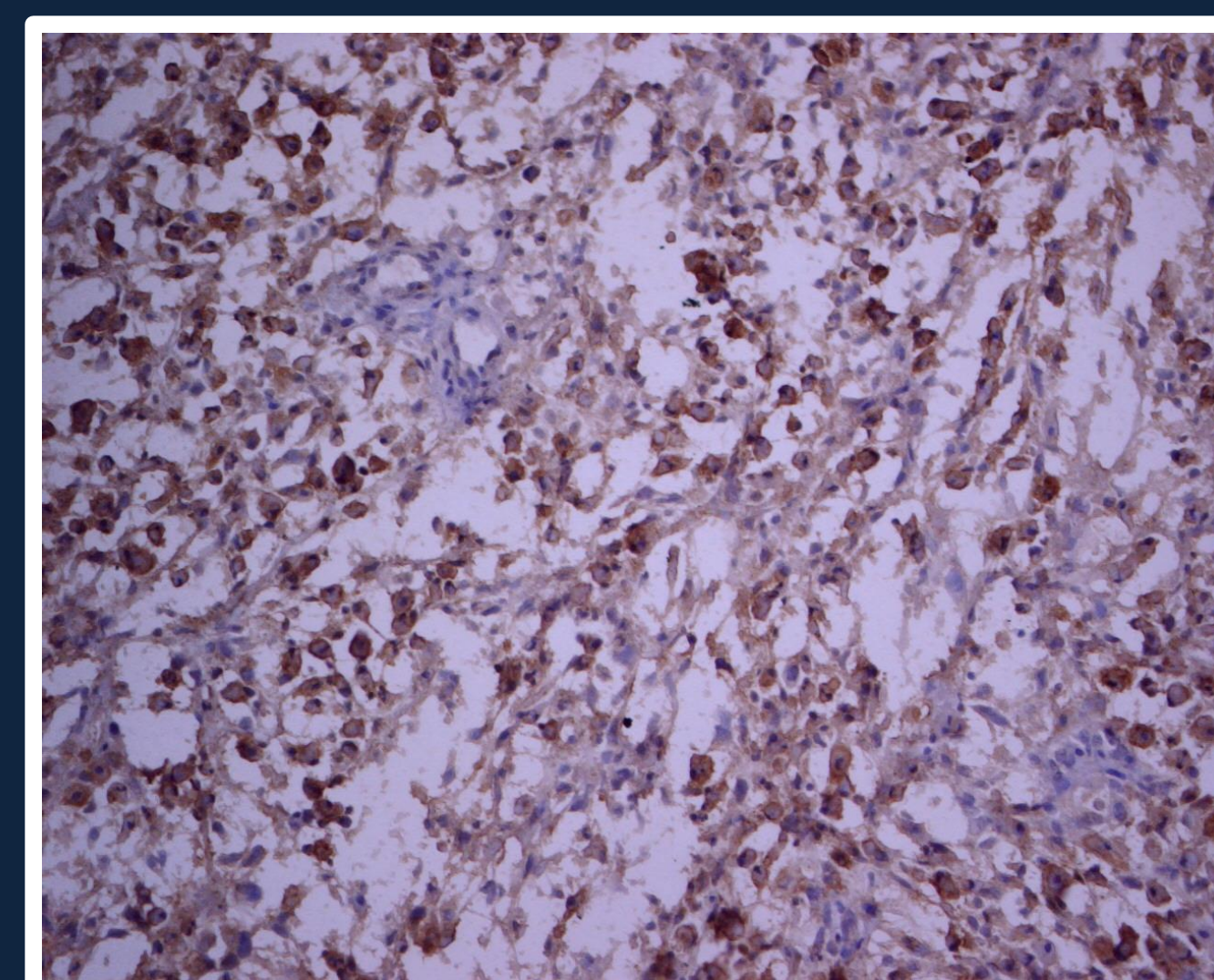
4. P53 and MDM2 expression results from immunohistochemistry



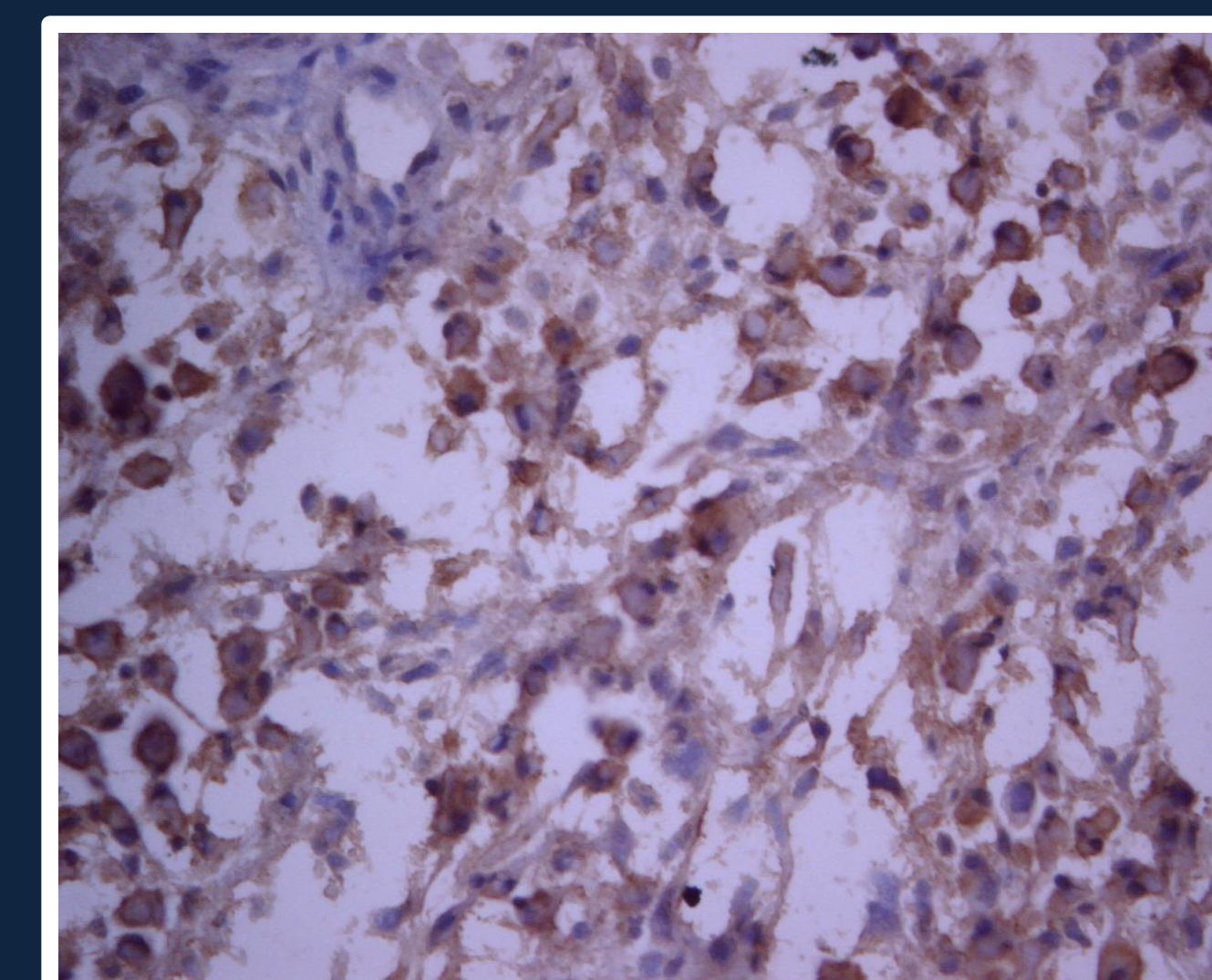
P53 positive ERMS 50% nuclear staining @ 20x magnification



P53 positive ERMS 50% nuclear staining @ 40x magnification



MDM2 positive ERMS 70% cytoplasm staining @ 20x magnification



MDM2 positive ERMS 70% cytoplasm staining @ 40x magnification

5. Taqman PCR genotyping for MDM2 Single Nucleotide Polymorphism (SNP 309)

Table 1: Mean Ages (in years) of Each Genotype for MDM2 SNP 309

| | ARMS | ERMS |
|--------------------|------|------|
| Mutant (m/m) | 8.29 | 15 |
| Heterozygous (w/m) | 2.66 | 9.14 |
| Wild Type (w/w) | 6.14 | 6.44 |

6. High expression of both p53 and MDM2 proteins was observed in ERMS tissues.

- High nuclear expression of p53 protein indicates accumulation of mutant p53 in the ERMS tumor cells.
- Wild type p53 has a very short half life (20 min), therefore no nuclear staining would be observed in cells with normal p53.
- High cytoplasmic MDM2 expression was observed in ERMS tumor cells.
- This is similar to observations in other cancer types, such as breast cancer, where high cytoplasmic MDM2 expression was associated with poor prognosis.

7. Future Directions

We showed that p53 and MDM2 pathways play an important role in RMS development, therefore any therapeutic agents targeting these pathways could be used for RMS treatment in the future. Further examination of these pathways in RMS can improve our understanding of risk classification and thereby influence management of this disease. We will analyze SNPs in p53 and MDM2 to further delineate the risk.

References:
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 Felix, C.A., Kappel, C.C., Mitsudomi, T., Nau, M.M., Tsokos, M., Crouch, G.D. et al. 1992. Frequency and diversity of p53 mutations in childhood rhabdomyosarcoma. *Cancer Res*, 52: 2243-2247.
 Nakajima, N., Ito, Y., Yokoyama, K., Uno, A., Kinukawa, N., Nemoto, N., and Moriyama, M. 2008. The expression of murine double minute two (MDM2) on *helicobacter pylori*-infected intestinal metaplasia and gastric cancer. *J Clin Biochem Nutr*, 44: 196-202.
 Rhabdomyosarcoma. <http://www.acor.org/ped-onc/diseases/rhabdo.html> (accessed 10/11/09).

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