Lauren Carr, Jessica Simendinger, Laura Vehring, Susan Kane, Katherine Crosby
Cell Signaling Technology, Inc., Danvers MA 01923

Visualization of Global Collagen in FFPE Tissues Using a Hydroxyproline Antibody

INTRODUCTION

Collagen is highly expressed in animal tissues and is the major component of the extracellular matrix (ECM). Dysregulation of collagen dynamics is implicated in many diseases, such as fibrosis and cancer, and the assessment of collagen while maintaining spatial context in tissues enables a better understanding of the role of collagen in disease. Used as a surrogate for collagen detection, hydroxyproline is a modified amino acid that is a major component of and is expressed almost exclusively in collagen. Here we describe a means of using a hydroxyproline antibody in paraffin-embedded tissues with a routine immunohistochemical assay that is easily implemented by any laboratory. Using the hydroxyproline antibody in the IHC assay, global collagen can be visualized in tissue in a species independent fashion, providing contextual information that is lost in traditional hydroxyproline assays. Additionally, the hydroxyproline antibody can be combined with other antibodies to enable detection of other markers of interest, something that is not easily achievable with the Sirius Red stain, a commonly used means of visualizing collagen in tissue samples.

METHODS

Single Stain: FFPE tissues were deparaffinized and rehydrated, subjected to antigen retrieval, then incubated with Hydroxyproline Antibody #73812 or COL1A1 (E8F4L) Rabbit mAb #72026 diluted in SignalStain® Antibody Diluent #8112 overnight at 4°C. Detection was performed using SignalStain® Boost IHC Detection Reagent (HRP, Rabbit) #8114 and SignalStain® DAB Substrate Kit #8059. Counterstaining was performed using Hematoxylin #14166.

Sirius Red Stain: FFPE tissues were deparaffinized and rehydrated, incubated with Picro-Sirius Red Solution (Abcam ab150681) for 1 hour at room temperature. Sections were rinsed in Acetic Acid Solution, followed by dehydration in 100% histology-grade alcohol. Note: Sirius Red labels collagen in red and muscle fibers/cytoplasm in yellow.

Dual Stain: Deparaffinized and rehydrated sections were subjected to antigen retrieval, then incubated with E-cadherin (4A2) MmAb #14472 and Hydroxyproline Antibody #73812 diluted in SignalStain® Antibody Diluent #8112 for 1 hour at room temperature. Detection was performed using SignalStain® Boost IHC Detection Reagent (HRP, Mouse) #8125 and DAB Substrate Kit #8059, followed by SignalStain® Boost IHC Detection Reagent (AP, Rabbit) #18653 and SignalStain® Vibrant Red Alkaline Phosphatase Substrate Kit #76713.

REFERENCES

1. Neuman, R. E. & Logan, M. A. The determination of hydroxyproline. J. Biol. Chem. 184, 299–306 (1950).

2. Cissell, D. D., Link, J. M., Hu, J. C. & Athanasiou, K. A. A Modified Hydroxyproline Assay Based on Hydrochloric Acid in Ehrlich's Solution Accurately Measures Tissue Collagen Content. Tissue Eng. Part C Methods 23, 243–250 (2017).

3. Gadd, V. L. Combining immunodetection with histochemical techniques: the effect of heat-induced antigen retrieval on picro-Sirius red staining. J. Histochem. Cytochem. 62, 902–906 (2014).

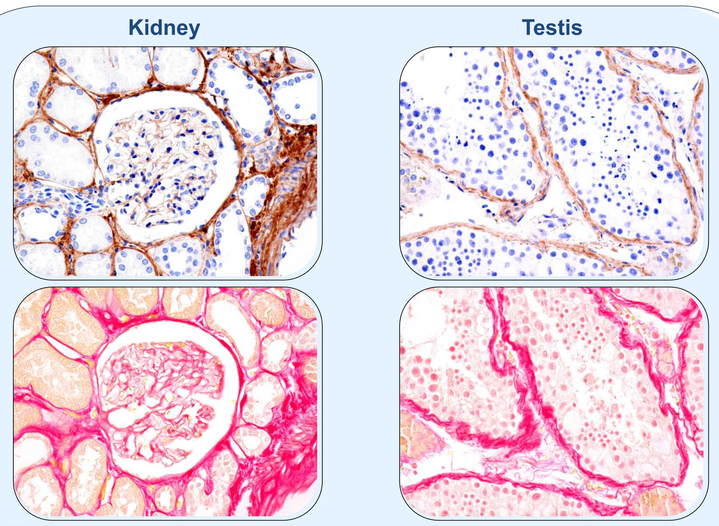

Figure 1. Normal human tissues stained with Hydroxyproline Antibody (top) or Sirius Red stain (bottom).

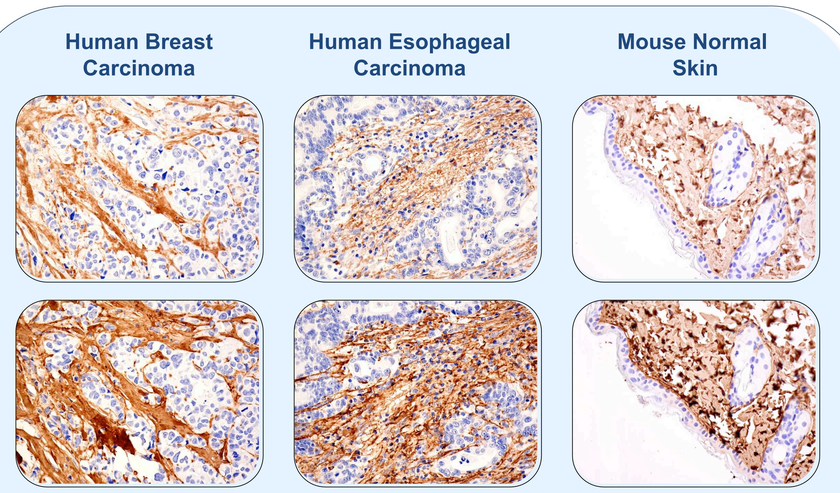

Figure 4. Human and mouse tissues stained with Hydroxyproline Antibody (top) or COL1A1 (E8F4L) Rabbit mAb (bottom).

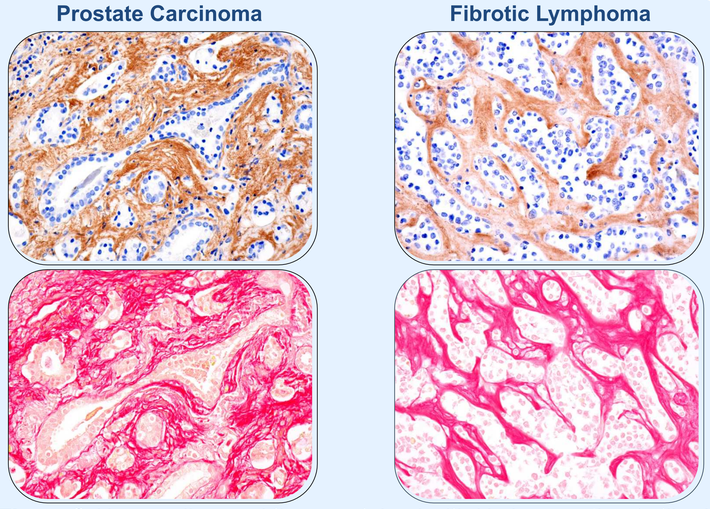

Figure 2. Human diseased tissues stained with Hydroxyproline Antibody (top) or Sirius Red stain (bottom).

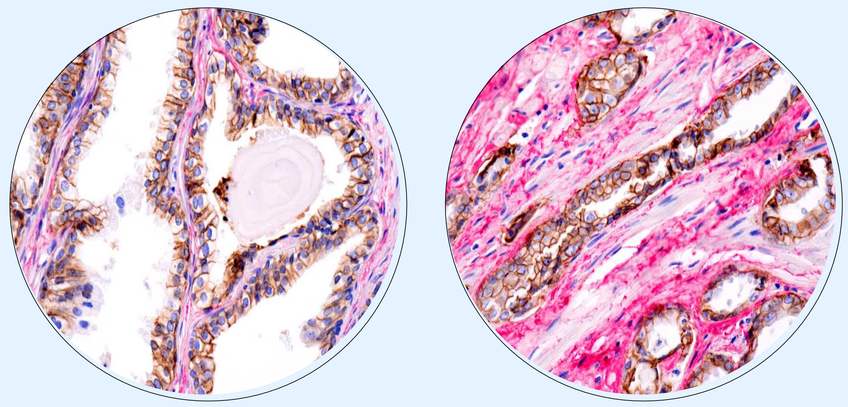

Figure 5. Dual immunohistochemical staining of paraffin-embedded human prostate carcinoma, 2 regions of interest, using E-cadherin (brown) and Hydroxyproline (red).

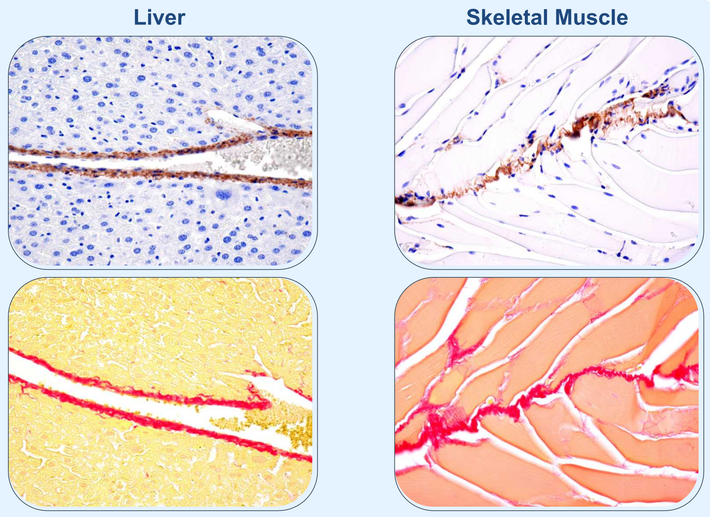

Figure 3. Normal mouse tissues stained with Hydroxyproline Antibody (top) or Sirius Red stain (bottom).

CONCLUSIONS

- **Hydroxyproline Antibody enables visualization of global collagen in FPPE tissue samples by a routine IHC assay, which may be preferable over a Sirius Red staining assay in which additional handling/disposal considerations are necessary.**
- **Hydroxyproline Antibody allows for species-independent detection of global collagen in FFPE tissues.**
- **Hydroxyproline Antibody can be combined with other antibodies to enable visualization of collagen along with other markers of interest.**

Cell Signaling TECHNOLOGY®

For Research Use Only. Not For Use in Diagnostic Procedures

Lauren Carr
email: lauren.carr@cellsignal.com
www.cellsignal.com/posters